

FIG. 1A

pET22b(+) forward primer:

5'-CGGGATCCT TCT GTT GAT CAC GGC TTC-3' (SEQ ID NO:3)

pET22b(+) reverse primer:

5'-CCCAAGCTT TGT TCT TCT CAT ACA GAC-3' (SEQ ID NO:4)

pPICZαA forward primer:

5'-TTCGGAATTC TCT GTT GAT CAC GGC TTC-3' (SEQ ID NO:15)

pPICZαA reverse primer:

5'-TGCTCTAGAGG TGT TCT TCT CAT ACA GAC TTG GCA-3' (SEQ ID NO:16)

5	10	15	20	25	30	35	40	45						
<u>tct gtt gat cac ggc ttc</u>	ctt	gtg	acc	agg	cat	agt	caa	aca	ata					
50	55	60	65	70	75	80	85	90						
gat	gac	cca	cag	tgt	cct	tct	ggg	acc	aaa	att	ctt	tac	cac	ggg
95	100	105	110	115	120	125	130	135						
tac	tct	ttg	ctc	tac	gtg	caa	ggc	aat	gaa	cgg	gcc	cat	gga	cag
140	145	150	155	160	165	170	175	180						
gac	ttg	ggc	acg	gcc	ggc	agc	tgc	ctg	cgc	aag	ttc	agc	aca	atg
185	190	195	200	205	210	215	220	225						
ccc	ttc	ctg	ttc	tgc	aat	att	aac	aac	gtg	tgc	aac	ttt	gca	tca
230	235	240	245	250	255	260	265	270						
cga	aat	gac	tac	tgc	tac	tgg	ctg	tcc	acc	cct	gag	ccc	atg	ccc
275	280	285	290	295	300	305	310	315						
atg	tca	atg	gca	ccc	atc	acg	ggg	gaa	aac	ata	aga	cca	ttt	att
320	325	330	335	340	345	350	355	360						
agt	agg	tgt	gct	gtg	tgt	gag	gcg	cct	gcc	atg	gtg	atg	gcc	gtg
365	370	375	380	385	390	395	400	405						
cac	agc	cag	acc	att	cag	atc	cca	ccg	tgc	ccc	agc	ggg	tgg	tcc
410	415	420	425	430	435	440	445	450						
tgc	ctg	tgg	atc	ggc	tac	tct	ttt	gtg	atg	cac	acc	agc	gct	ggt
455	460	465	470	475	480	485	490	495						
gca	gaa	ggc	tct	ggc	caa	gcc	ctg	gcg	tcc	ccc	ggc	tcc	tgc	ctg
500	505	510	515	520	525	530	535	540						
gag	gag	ttt	aga	agt	gcg	cca	ttc	atc	gag	tgt	cac	ggc	cgt	ggg
545	550	555	560	565	570	575	580	585						
acc	tgc	aat	tac	tac	gca	aac	gct	tac	agc	ttt	tgg	ctc	gcc	acc
590	595	600	605	610	615	620	625	630						
ata	gag	agg	agc	gag	atg	ttc	aag	aag	cct	acg	ccg	tcc	acc	ttg
635	640	645	650	655	660	665	670	675						
aag	gca	ggg	gag	ctg	cgc	acg	cac	gtc	agc	cgc	<u>tgc</u>	<u>caa</u>	<u>gtc</u>	<u>tgt</u>
680	685	690												
<u>atg</u>	<u>aga</u>	<u>aga</u>	<u>aca</u>	taa	(SEQ ID NO:1)									

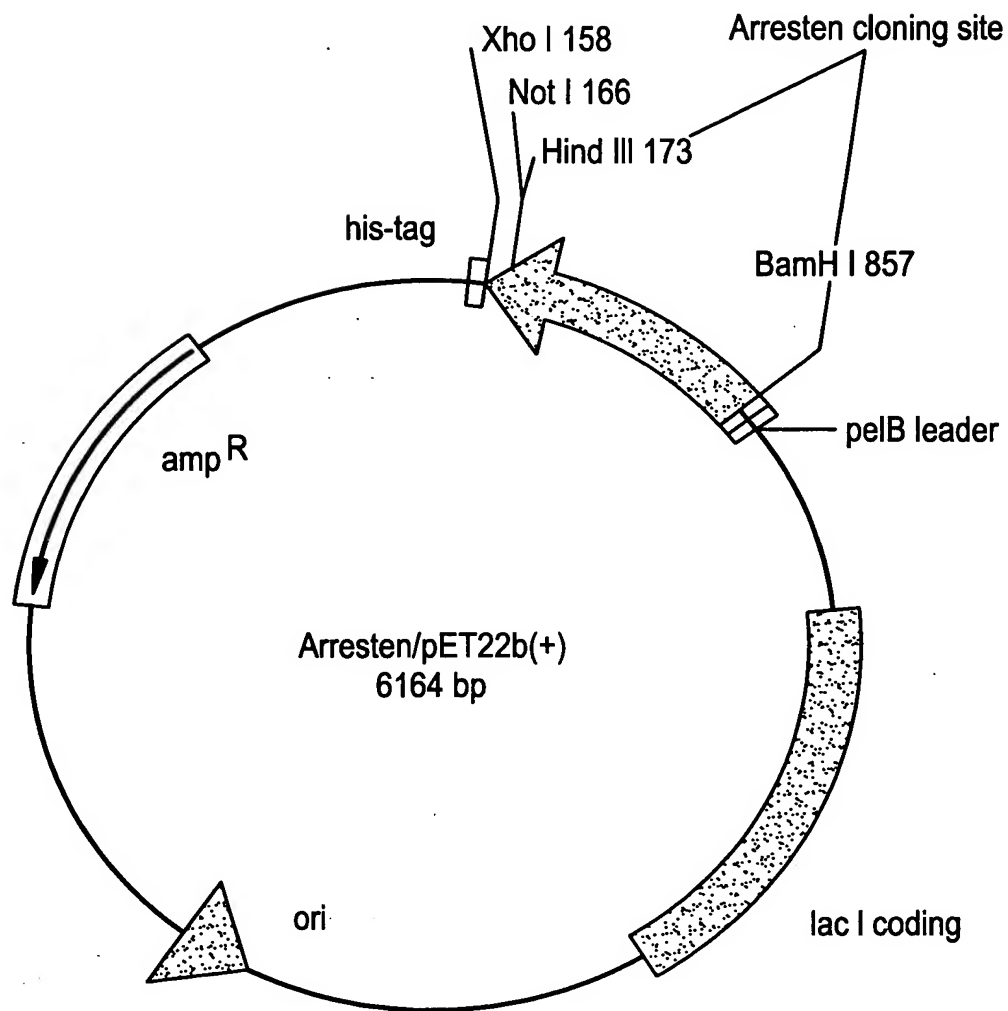
FIG. 1B

5	10	15	20	25	30	35	40	45						
SVD	HGF	LVT	RHS	QTI	DDP	QCP	SGT	KIL	YHG	YSL	LYV	QGN	ERA	HGQ
50	55	60	65	70	75	80	85	90						
DLG	TAG	SCL	RKF	STM	PFL	FCN	INN	VCN	FAS	RND	YSY	WLS	TPE	PMP
95	100	105	110	115	120	125	130	135						
MSM	API	TGE	NIR	PFI	SRC	AVC	EAP	AMV	MAV	HSQ	TIQ	IPP	CPS	GWS
140	145	150	155	160	165	170	175	180						
SLW	IGY	SFV	MHT	SAG	AEG	SGQ	ALA	SPG	SCL	EEF	RSA	PFI	ECH	GRG
185	190	195	200	205	210	215	220	225						
TCN	YYA	NAY	SFW	LAT	IER	SEM	FKK	PTP	STL	KAG	ELR	THV	SRC	QVC

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MRR T (SEQ ID NO:2)

FIG. 2



Forward primer: 5'-cgggatccttctgttgatcacggcttc-3'

Reverse primer: 5'-cccaagcttgttcttctacacagac-3'

FIG. 3A

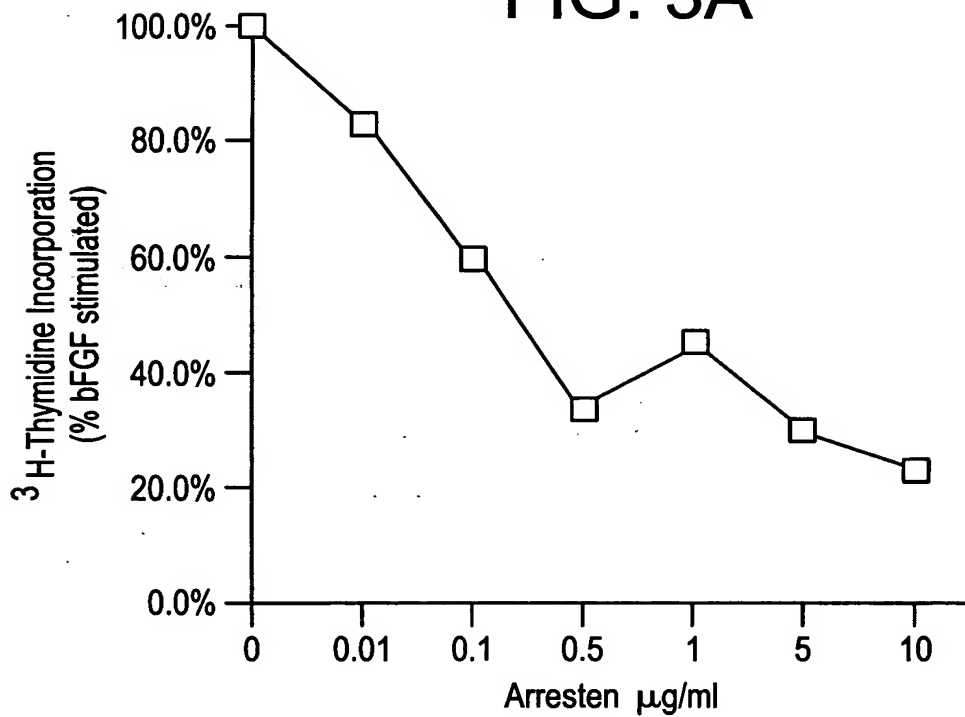


FIG. 3B

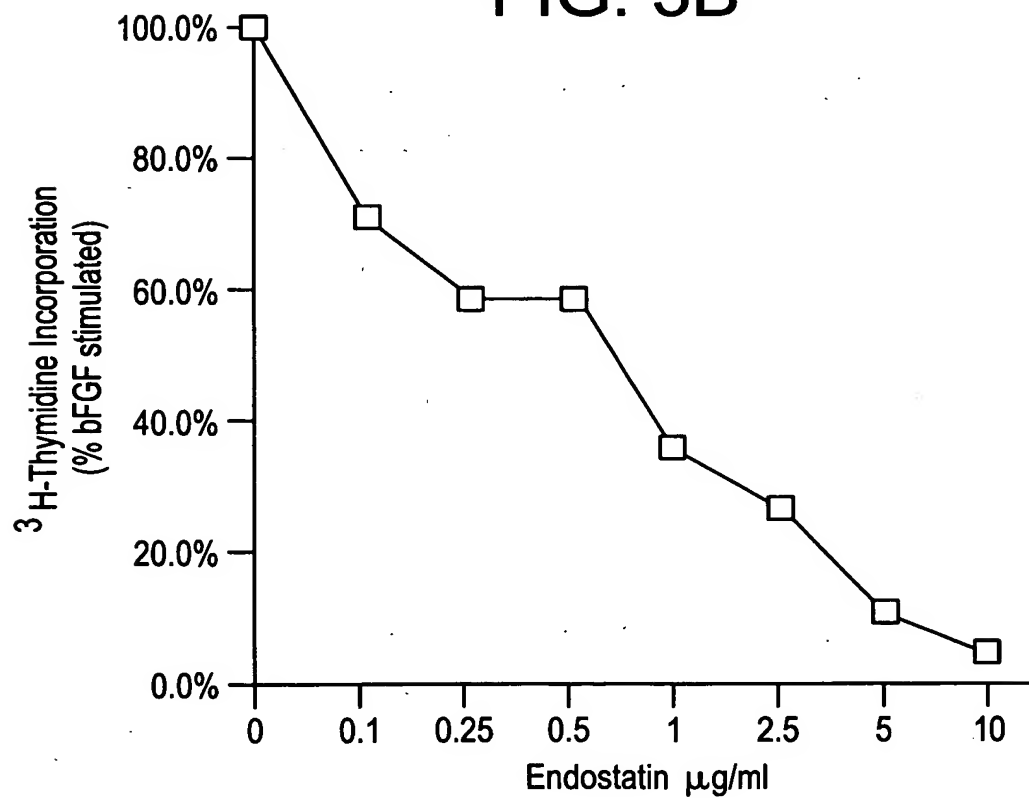


FIG. 4A

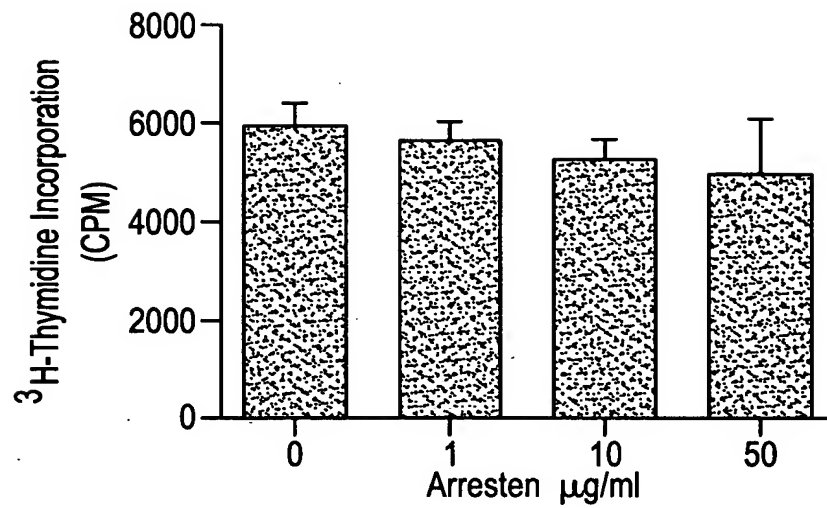


FIG. 4B

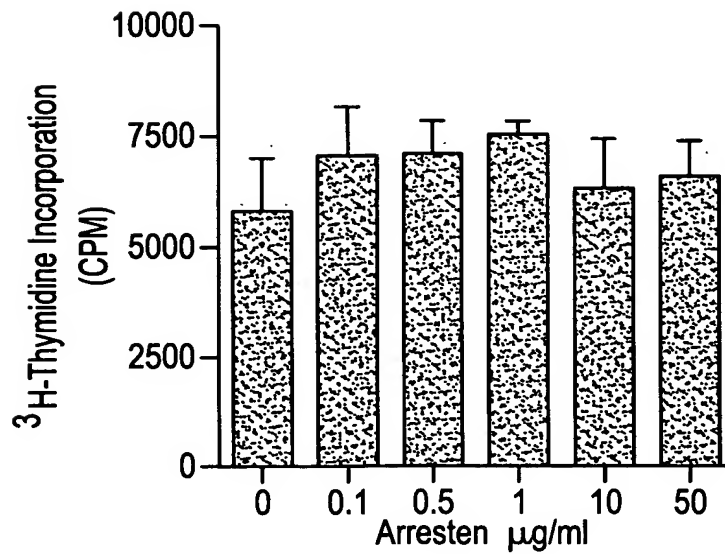


FIG. 4C

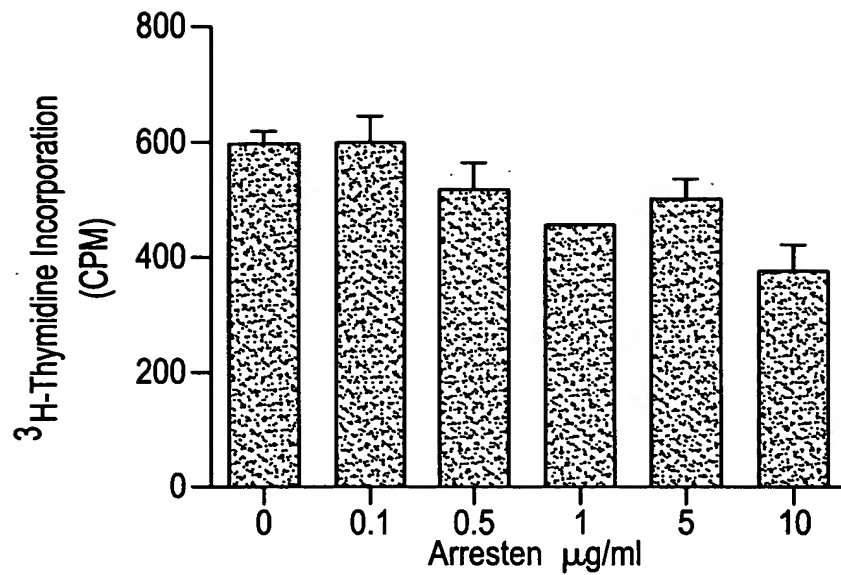


FIG. 4D

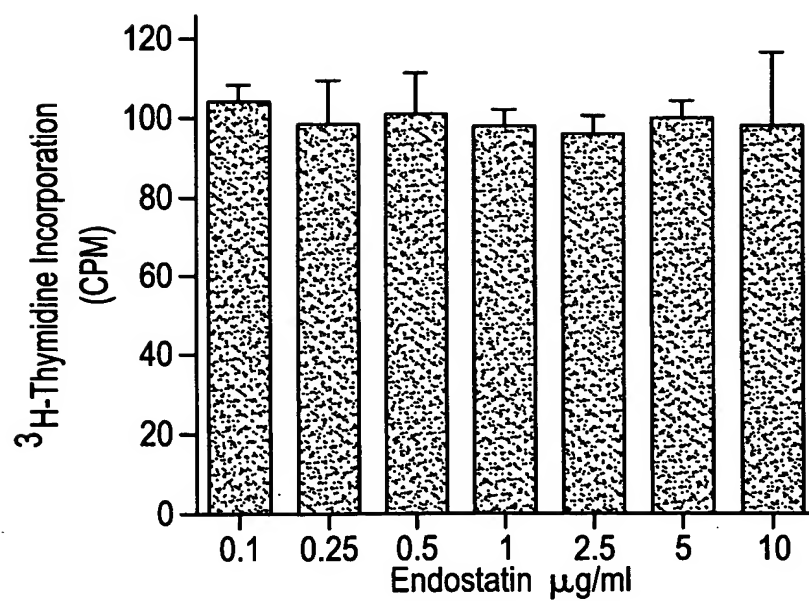


FIG. 5A

Control

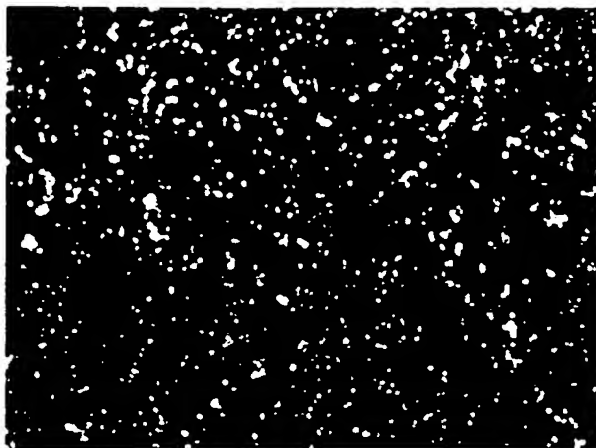


FIG. 5B

Arresten 2 μ g/ml

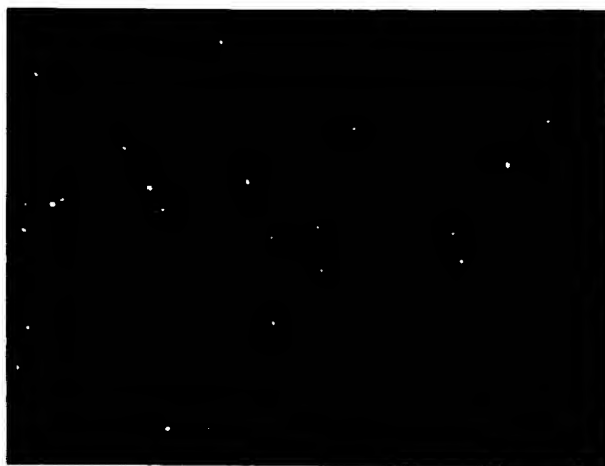


FIG. 5C

Endostatin 20 μ g/ml

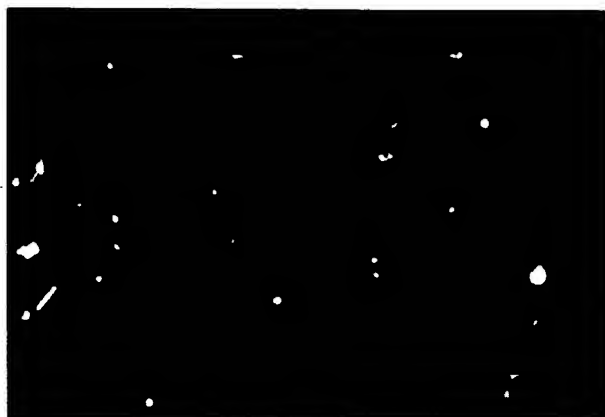


FIG. 6

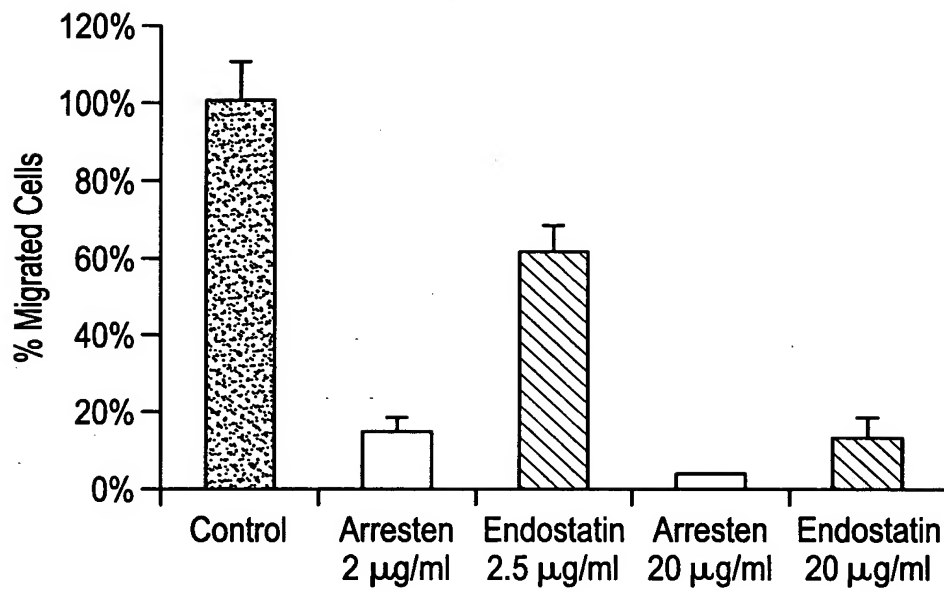


FIG. 7

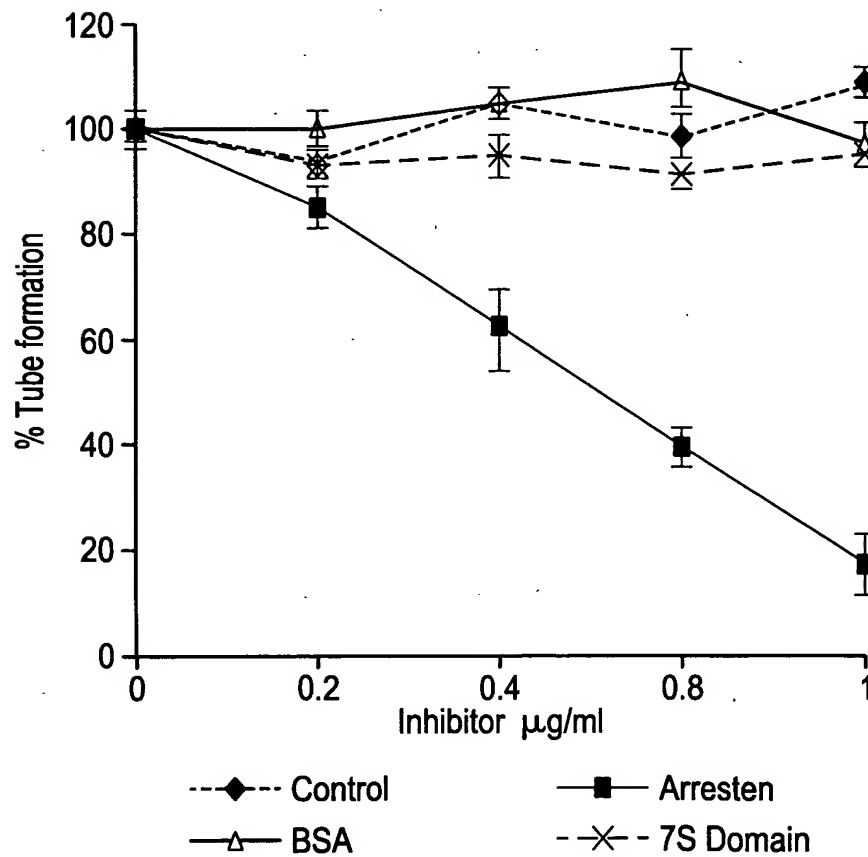


FIG. 8A

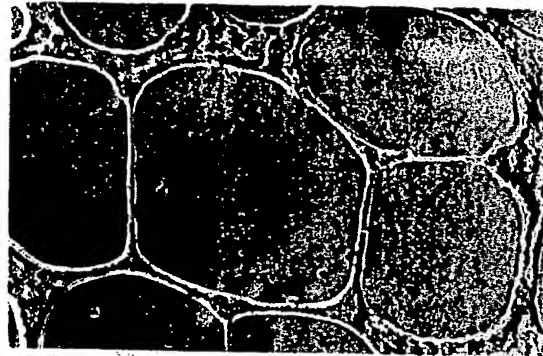


FIG. 8B



FIG. 9A

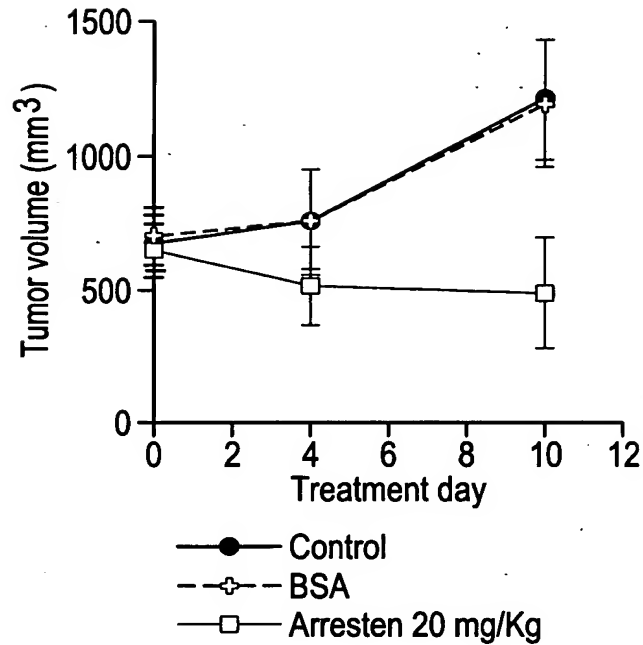


FIG. 9B

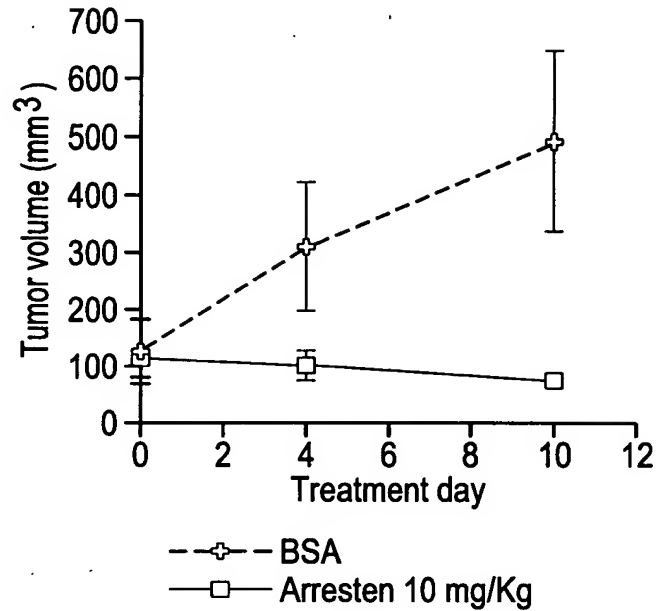


FIG. 9C

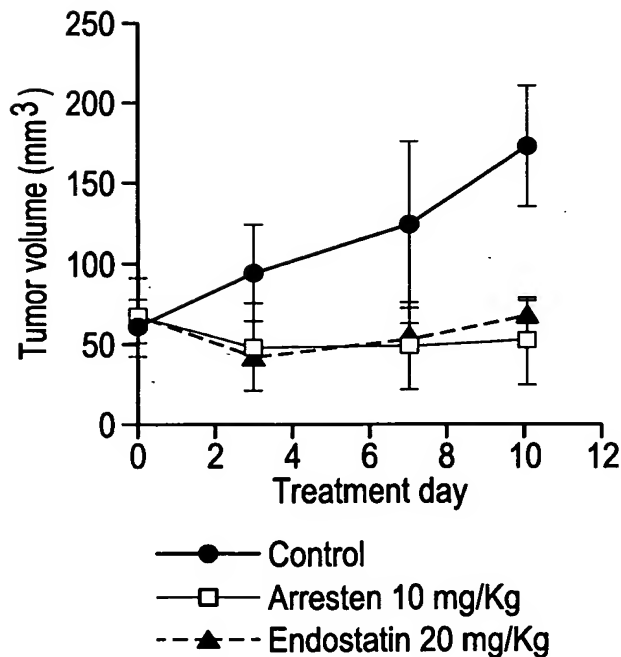
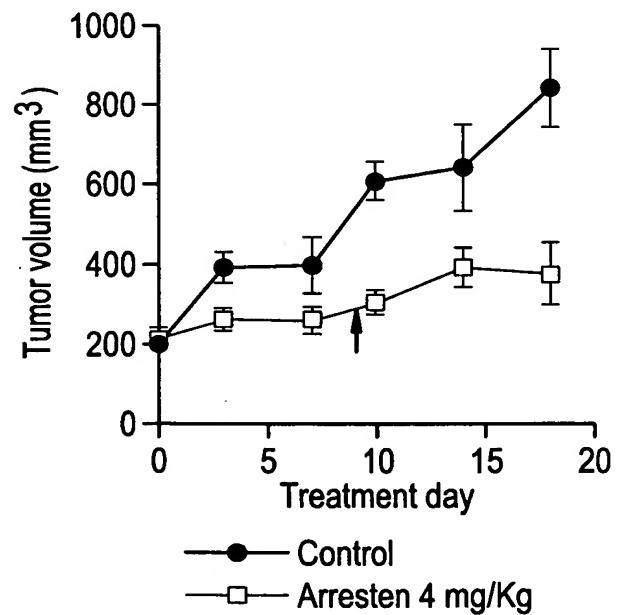


FIG. 9D



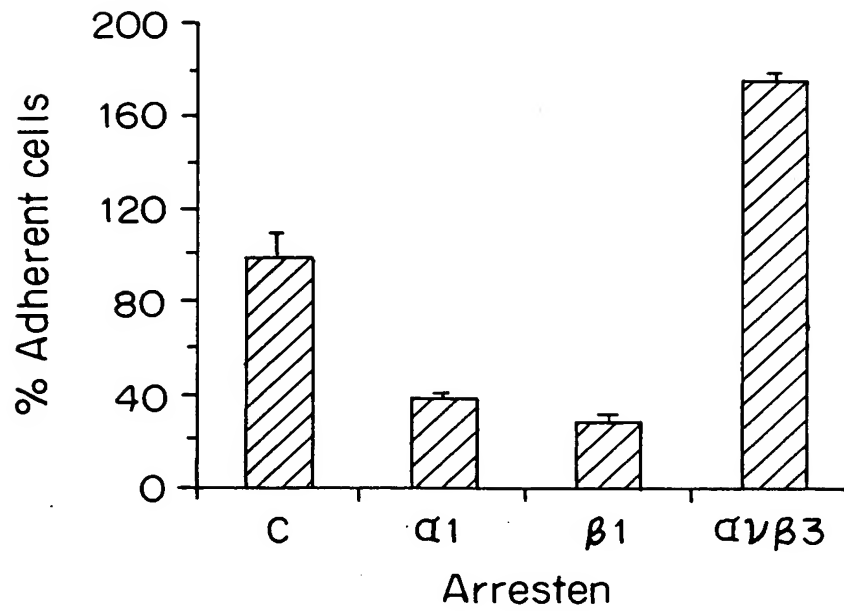


FIG. IOA

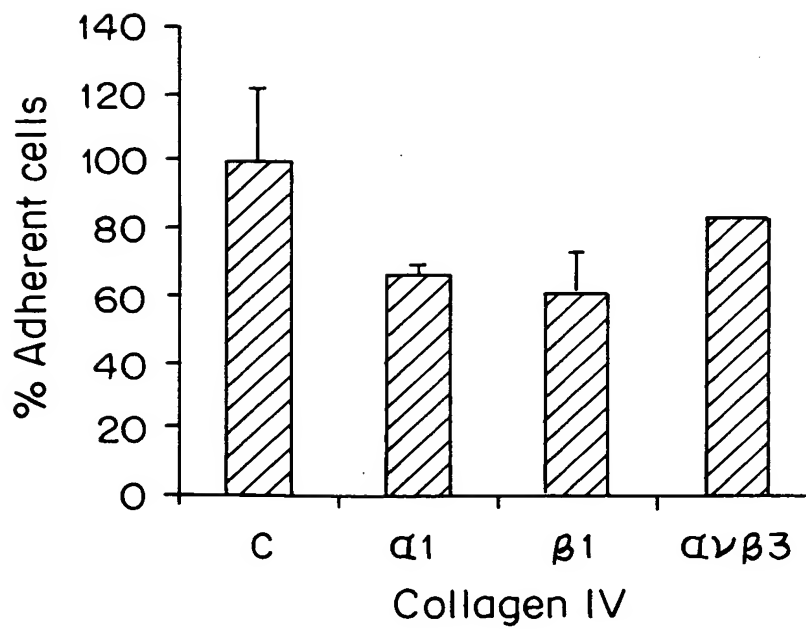


FIG. IOB

FIG. 11A

pET22b(+) forward primer:

5'-CGGGATCCT GTC AGC ATC GGC TAC CTC-3' (SEQ ID NO:7)

pET22b(+) reverse primer:

5'-CCCAAGCTT CAG GTT CTT CAT GCA CAC-3' (SEQ ID NO:8)

pPICZαA forward primer:

5'-TTCGGAATTC GTC AGC ATC GGC TAC CTC CTG-3' (SEQ ID NO:17)

pPICZαA reverse primer:

5'-GGGGTACCCC CAG GTT CTT CAT GCA CAC CTG G-3' (SEQ ID NO:18)

5	10	15	20	25	30	35	40	45						
<u>gtc agc atc ggc tac ctc</u>	<u>ctg</u>	gtg	aag	cac	agc	cag	acg	gac	cag					
50	55	60	65	70	75	80	85	90						
gag	ccc	atg	tgc	ccg	gtg	ggc	atg	aac	aaa	ctc	tgg	agt	gga	tac
95	100	105	110	115	120	125	130	135						
agc	ctg	ctg	tac	ttc	gag	ggc	cag	gag	aag	gcg	cac	aac	cag	gac
140	145	150	155	160	165	170	175	180						
ctg	ggg	ctg	gcg	ggc	tcc	tgc	ctg	gcg	cgg	ttc	agc	acc	atg	ccc
185	190	195	200	205	210	215	220	225						
ttc	ctg	tac	tgc	aac	cct	ggt	gat	gtc	tgc	tac	tat	gcc	agc	cgg
230	235	240	245	250	255	260	265	270						
aac	gac	aag	tcc	tac	tgg	ctc	tct	acc	act	gcg	ccg	ctg	ccc	atg
275	280	285	290	295	300	305	310	315						
atg	ccc	gtg	gcc	gag	gac	gag	atc	aag	ccc	tac	atc	agc	cgc	tgt
320	325	330	335	340	345	350	355	360						
tct	gtg	tgt	gag	gcc	ccg	gcc	atc	gcc	atc	gcg	gtc	cac	agt	cag
365	370	375	380	385	390	395	400	405						
gat	gtc	tcc	atc	cca	cac	tgc	cca	gct	ggg	tgg	cgg	agt	ttg	tgg
410	415	420	425	430	435	440	445	450						
atc	gga	tat	tcc	ttc	ctc	atg	cac	acg	gcg	gcg	gga	gac	gaa	ggc
455	460	465	470	475	480	485	490	495						
ggg	ggc	caa	tca	ctg	gtg	tca	ccg	ggc	agc	tgt	cta	gag	gac	ttc
500	505	510	515	520	525	530	535	540						
cgc	gcc	aca	cca	ttc	atc	gaa	tgc	aat	gga	ggc	cgc	ggc	acc	tgc
545	550	555	560	565	570	575	580	585						
cac	tac	tac	gcc	aac	aag	tac	agc	ttc	tgg	ctg	acc	acc	att	ccc
590	595	600	605	610	615	620	625	630						
gag	cag	agc	ttc	cag	ggc	tgc	ccc	tcc	gcc	gac	acg	ctc	aag	gcc
635	640	645	650	655	660	665	670	675						
ggc	ctc	atc	cgc	aca	cac	atc	agc	cgc	tgc	<u>cag</u>	<u>gtg</u>	<u>tgc</u>	<u>atg</u>	<u>aag</u>
680														
<u>aac</u>	<u>ctg</u>	tga												

(SEQ ID NO:5)

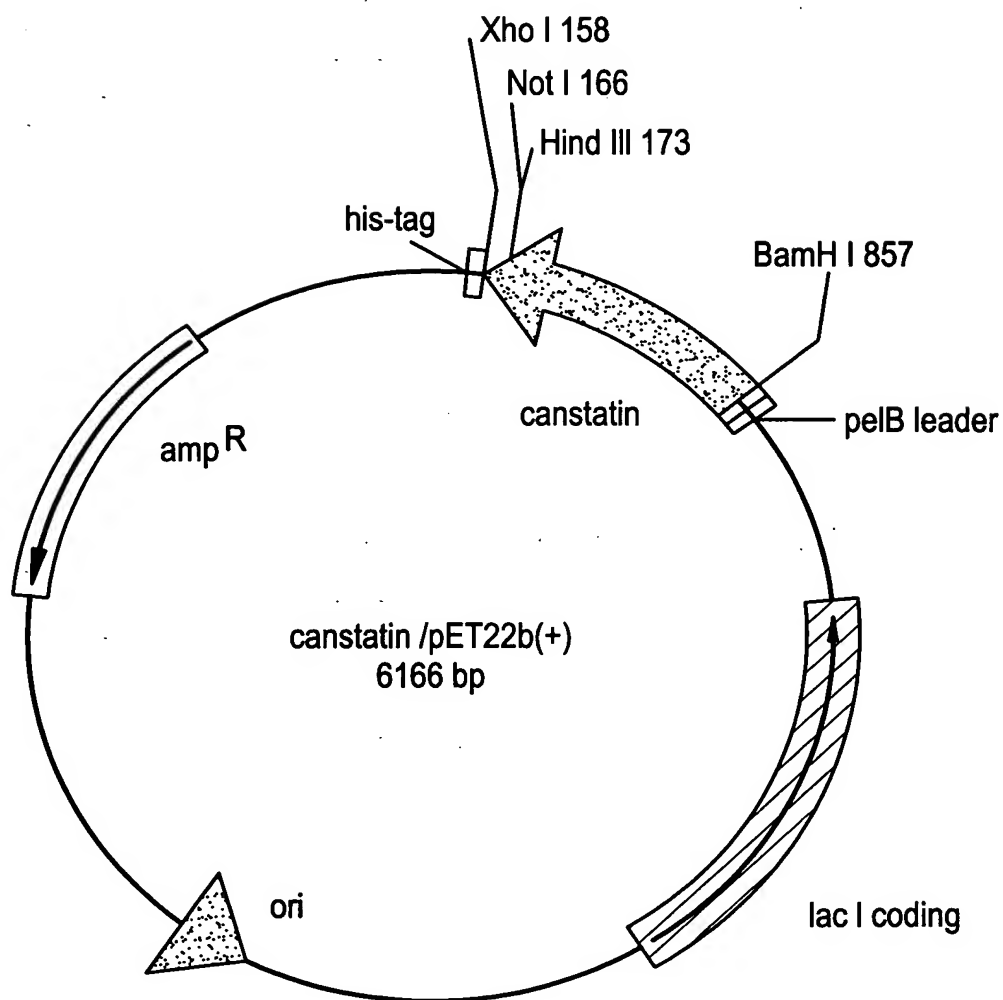
FIG. 11B

5	10	15	20	25	30	35	40	45						
VSI	GYL	LVK	HSQ	TDQ	EPM	CPV	GMN	KLW	SGY	SLL	YFE	GQE	KAH	NQD
50	55	60	65	70	75	80	85	90						
LGL	AGS	CLA	RFS	TMP	FLY	CNP	GDV	CYY	ASR	NDK	SYW	LST	TAP	LPM
95	100	105	110	115	120	125	130	135						
MPV	AED	EIK	PYI	SRC	SVC	EAP	AIA	IAV	HSQ	DVS	IPH	CPA	GWR	SLW
140	145	150	155	160	165	170	175	180						
IGY	SFL	MHT	AAG	DEG	GGQ	SLV	SPG	SCL	EDF	RAT	PFI	ECN	GGR	GTC
185	190	195	200	205	210	215	220	225						
HYY	ANK	YSF	WLT	TIP	EQS	FQG	SPS	ADT	LKA	GLI	RTH	ISR	CQV	CMK

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NL (SEQ ID NO:6)

FIG. 12



Forward primer: 5'-cgggatcctgtcagcatcggtacctc-3'

Reverse primer: 5'-cccaagcttcaggttctcatgcacac-3'

FIG. 13A

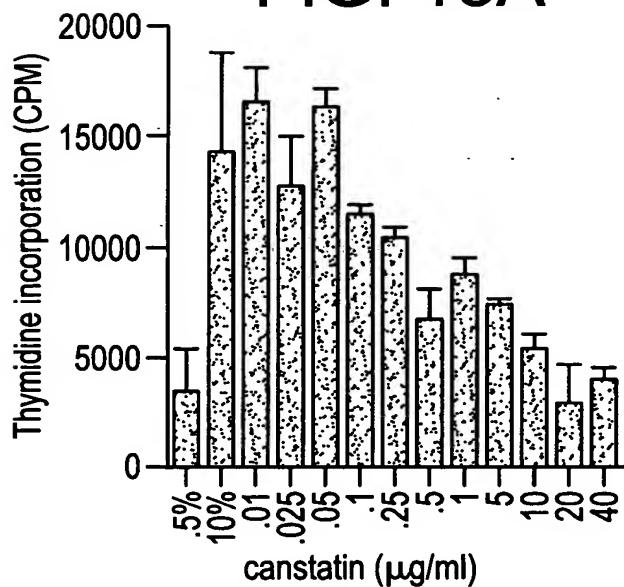


FIG. 13B

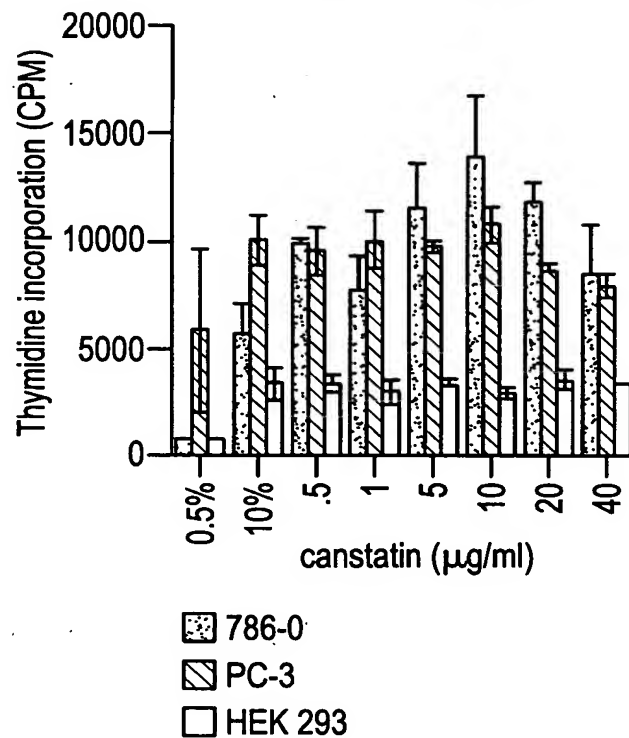


FIG. 13C

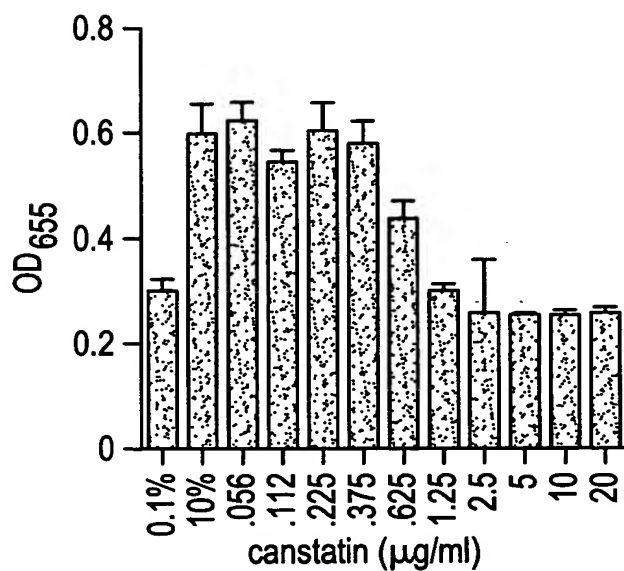


FIG. 13D

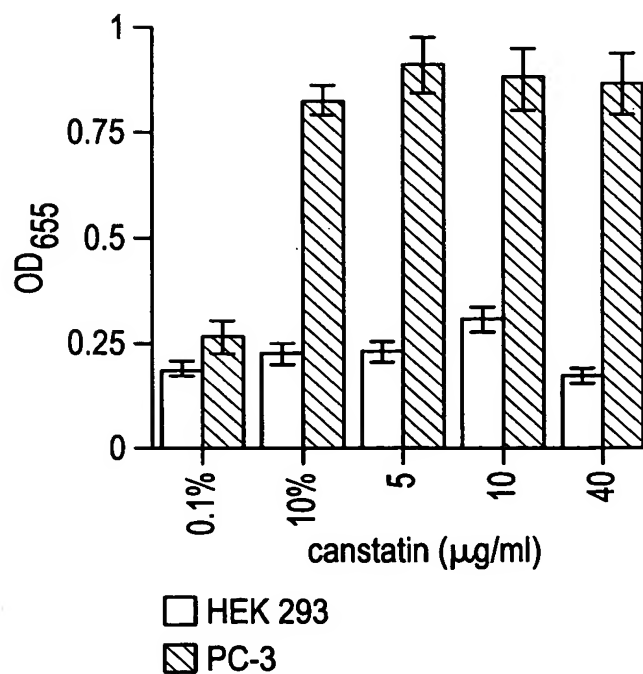


FIG. 14

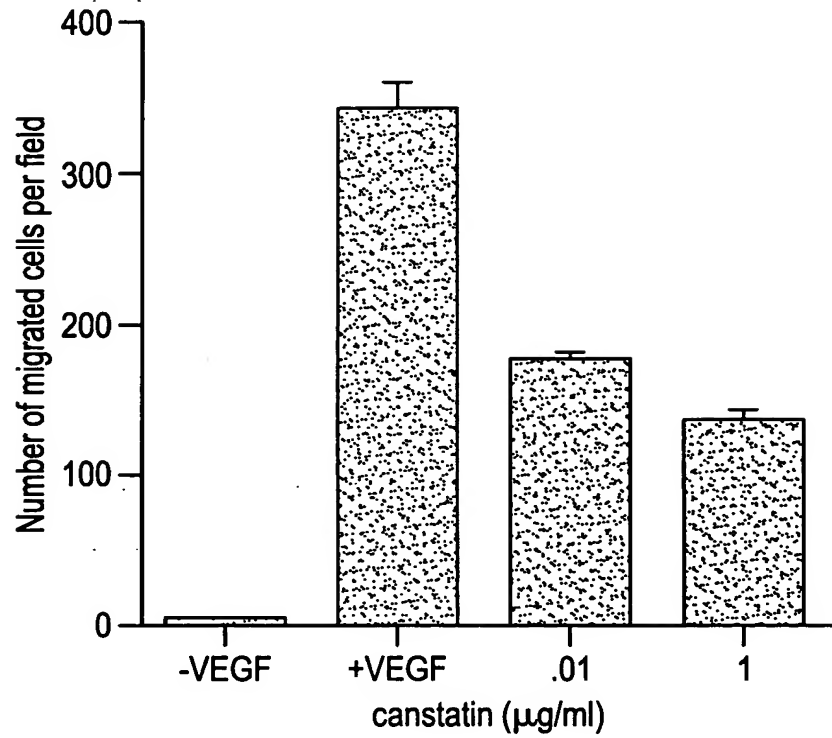
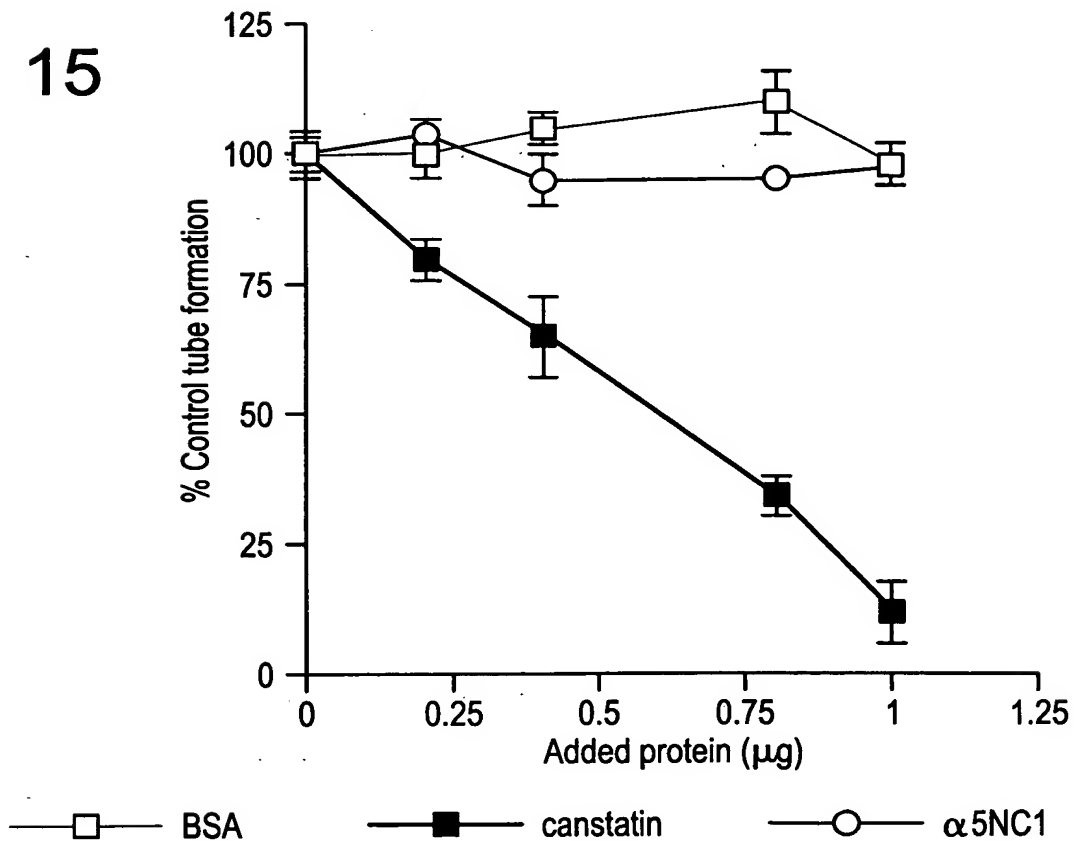


FIG. 15



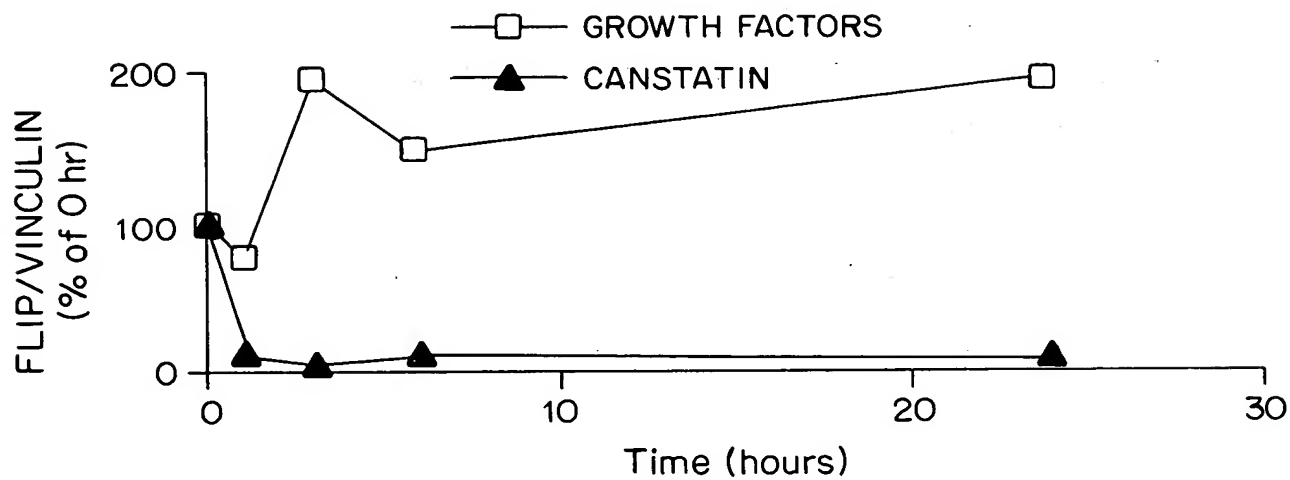


FIG. 16

FIG. 17A

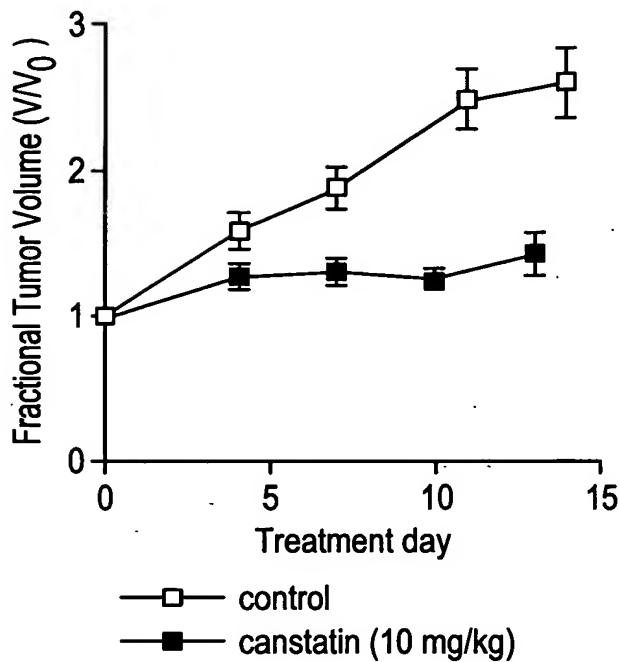


FIG. 17B

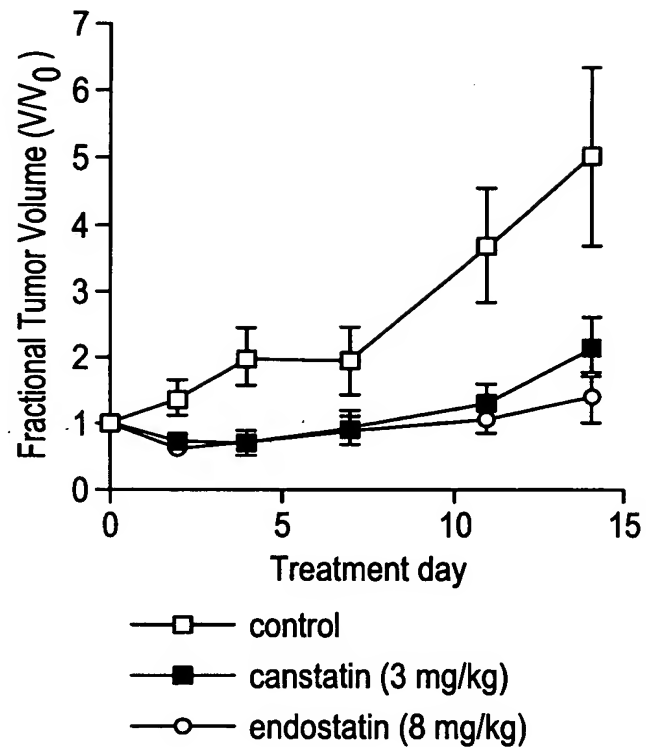


FIG. 17C

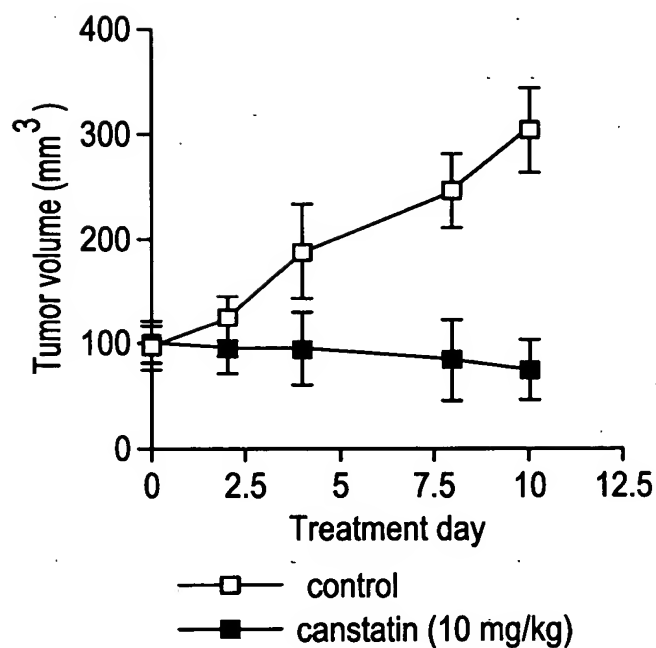


FIG. 17D

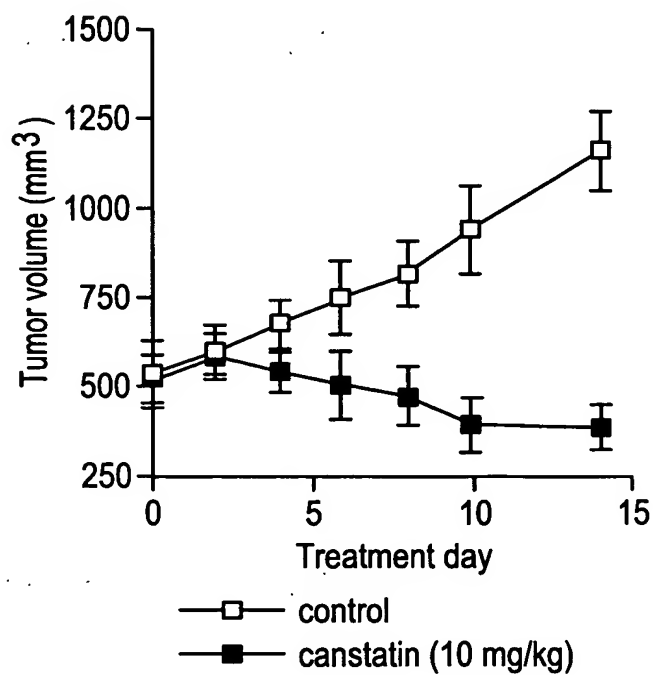


FIG. 18A

pET22b(+) forward primer:

5'-CGGGAT CCA GGT TTG AAA GGA AAA CGT-3' (SEQ ID NO:11)

pET22b(+) reverse primer:

5'-CCCAAGCTT TCA GTG TCT TTT CTT CAT-3' (SEQ ID NO:12)

5	10	15	20	25	30	35	40	45
<u>cca ggt ttg aaa gga aaa cgt</u>	gga gac agt	gga tca cct	gca acc					
50	55	60	65	70	75	80	85	90
tgg aca acg	aga ggc ttt	gtc ttc acc	cga cac agt	caa acc	aca			
95	100	105	110	115	120	125	130	135
gca att cct	tca tgt cca	gag ggg aca	gtg cca ctc	tac agt	ggg			
140	145	150	155	160	165	170	175	180
ttt tct ttt	ctt ttt gta	caa gga aat	caa cga gcc	cac gga	caa			
185	190	195	200	205	210	215	220	225
gac ctt gga	act ctt ggc	agc tgc ctg	cag cga ttt	acc aca	atg			
230	235	240	245	250	255	260	265	270
cca ttc tta	ttc tgc aat	gtc aat gat	gta tgt aat	ttt gca	tct			
275	280	285	290	295	300	305	310	315
cga aat gat	tat tca tac	tgg ctg tca	aca cca gct	ctg atg	cca			
320	325	330	335	340	345	350	355	360
atg aac atg	gct ccc att	act ggc aga	gcc ctt	gag cct	tat ata			
365	370	375	380	385	390	395	400	405
agc aga tgc	act gtt tgt	gaa ggt cct	gcg atc	gcc ata	gcc gtt			
410	415	420	425	430	435	440	445	450
cac agc caa	acc act gac	att cct cca	tgt cct	cac ggc	tgg att			
455	460	465	470	475	480	485	490	495
tct ctc tgg	aaa gga ttt	tca ttc atc	atg ttc	aca agt	gca ggt			
500	505	510	515	520	525	530	535	540
tct gag ggc	acc ggg caa	gca ctg gcc	tcc cct	ggc tcc	tgc ctg			
545	550	555	560	565	570	575	580	585
gaa gaa ttc	cga gcc agc	cca ttt cta	gaa tgt	cat gga	aga gga			
590	595	600	605	610	615	620	625	630
acg tgc aac	tac tat tca	aat tcc tac	agt ttc	tgg ctg	gct tca			
635	640	645	650	655	660	665	670	675
tta aac cca	gaa aga atg	ttc aga	aag cct	att cca	tca act	gtg		
680	685	690	695	700	705	710	715	720
aaa gct ggg	gaa tta gaa	aaa ata	ata agt	cgc tgt	cag gtg	tgc		
725	730	735						
<u>atg aag aaa aga cac tga</u>								

(SEQ ID NO:9)

pET22b- α 3(IV) NC1 = nucleotides 4 through 735

Tumstatin 333 = nucleotides 4 through 375

Tumstatin 334 - nucleotide 376 through 735

FIG. 18B

*
5 10 15 20 25 30 35 40 45
PGL KGK RGD SGS PAT WTT RGF VFT RHS QTT AIP SCP EGT VPL YSG
50 55 60 65 70 75 80 85 90
FSF LFV QGN QRA HGQ DLG TLG SCL QRF TTM PFL FCN VND VCN FAS
95 100 105 110 115 120 125 130 135
RND YSY WLS TPA LMP MNM API TGR ALE PYI SRC TVC EGP AIA IAV
140 145 150 155 160 165 170 175 180
HSQ TTD IPP CPH GWI SLW KGF SFI MFT SAG SEG TGQ ALA SPG SCL
185 190 195 200 205 210 215 220 225
EEF RAS PFL ECH GRG TCN YYS NSY SFW LAS LNP ERM FRK PIP STV
230 235 240 245
KAG ELE KII SRC QVC MKK RH (SEQ ID NO:10)

pET22b $\alpha 3$ (IV) NC1 = residues 2 through 245

Tumstatin 333 = residues 2 through 125

Tumstatin 334 = residues 126 through 245

FIG. 19

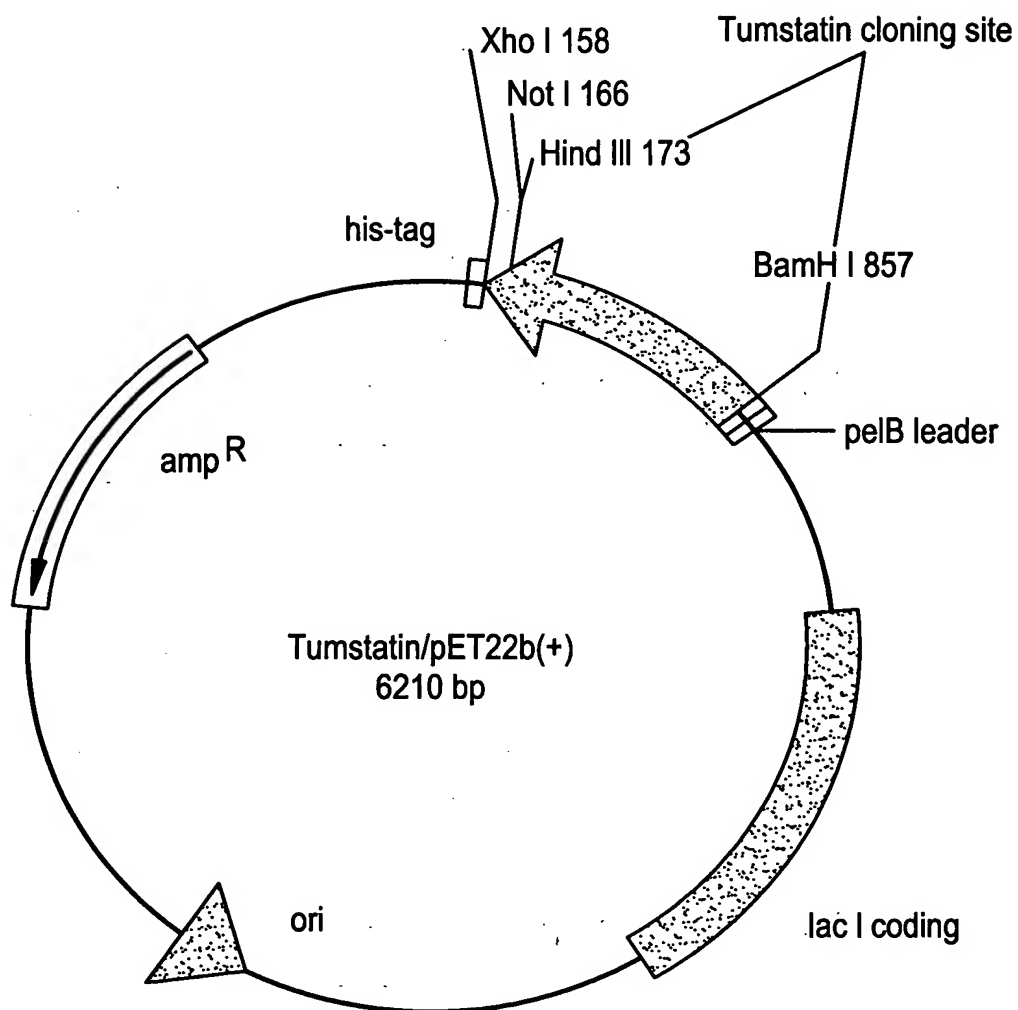


FIG. 20

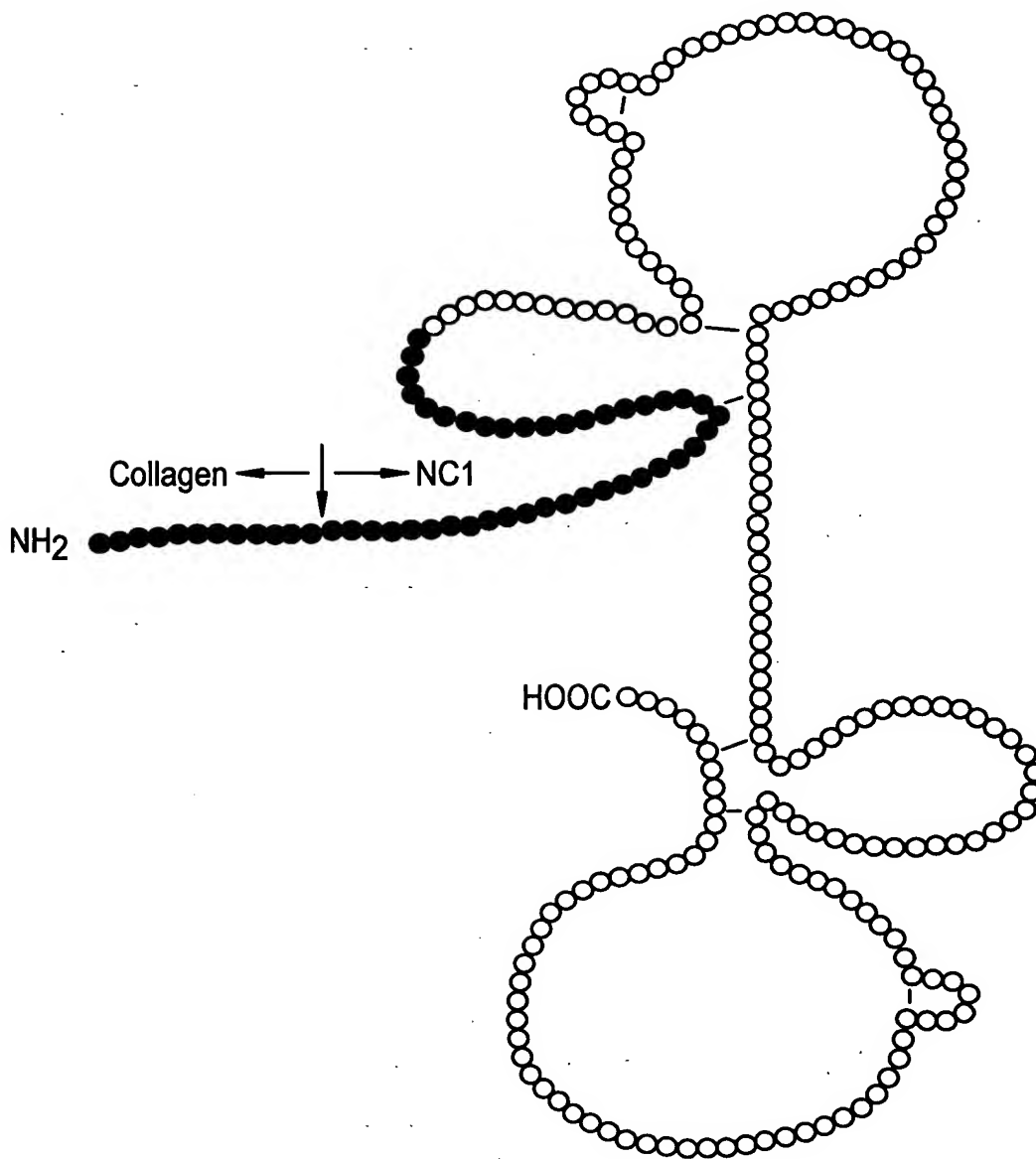


FIG. 21C

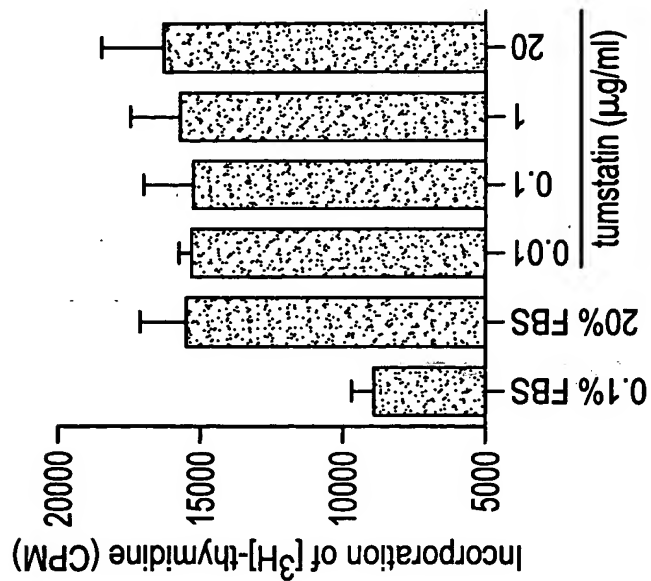


FIG. 21B

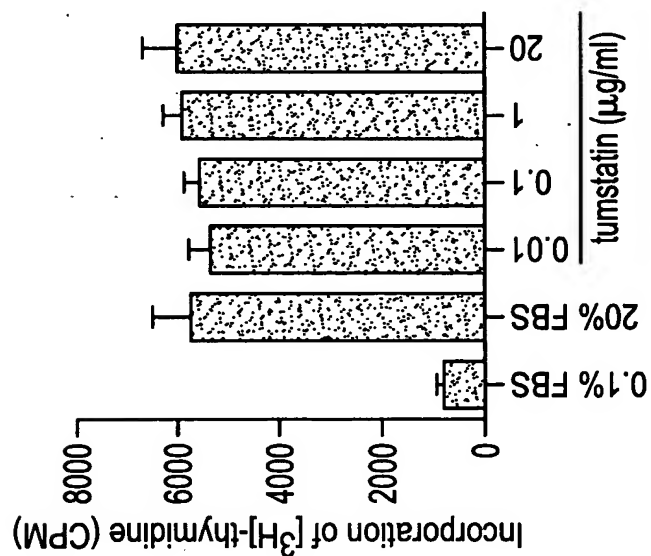
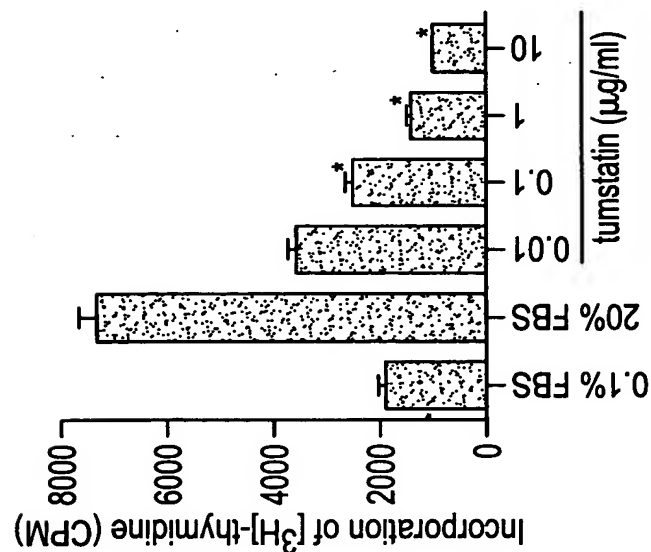


FIG. 21A



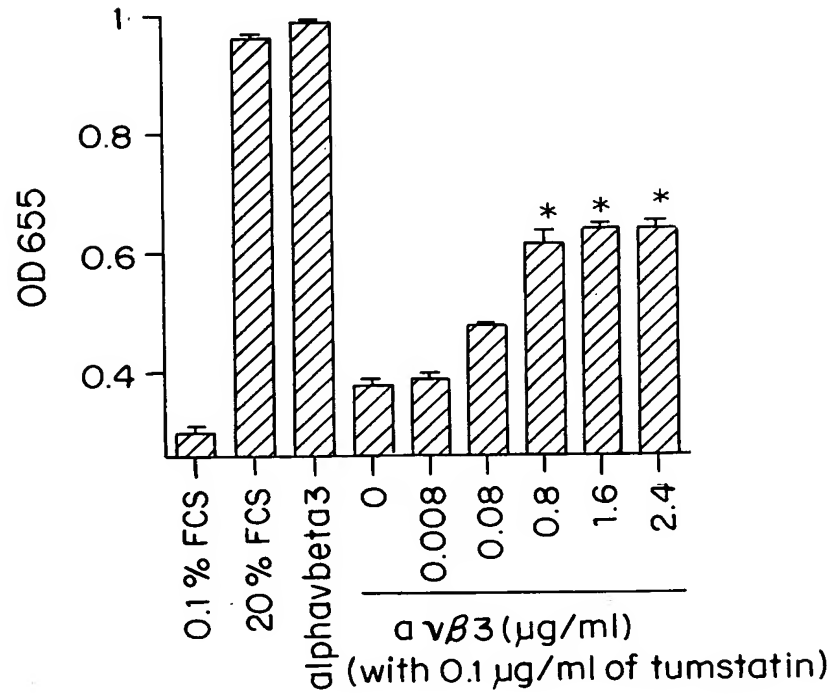


FIG. 22

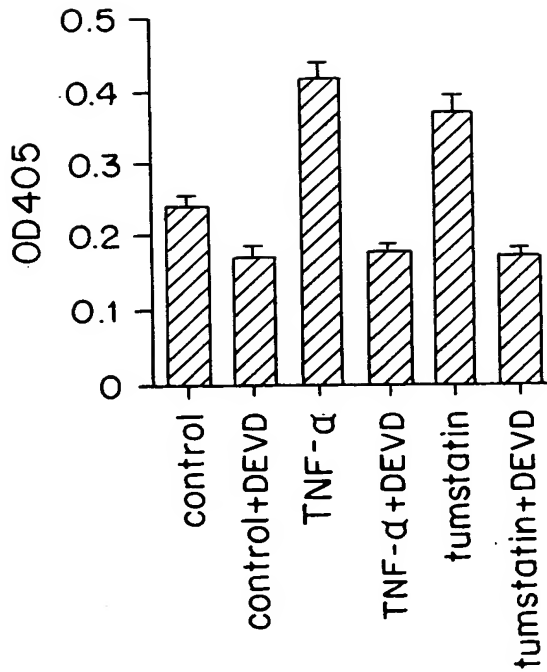


FIG. 23A

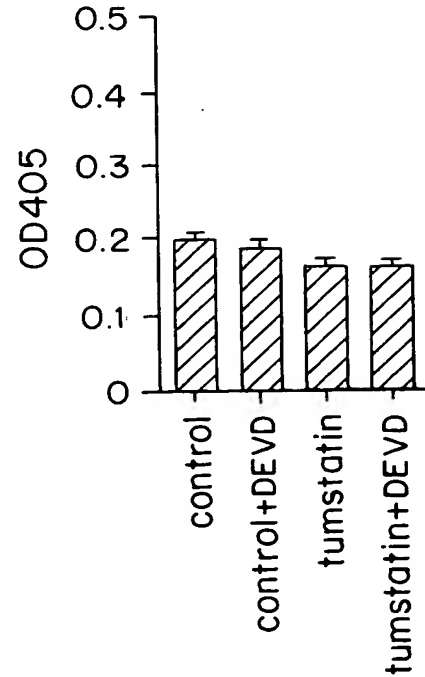


FIG. 23B

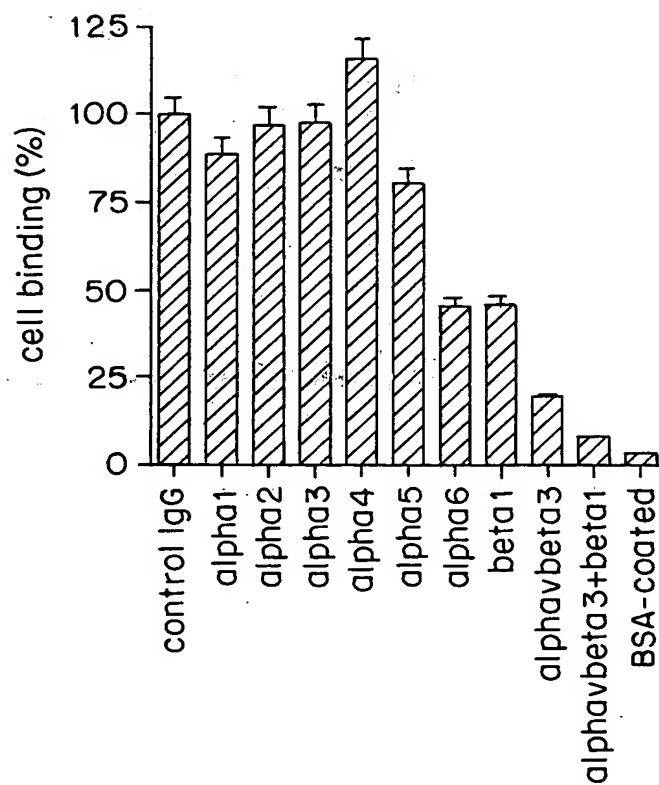


FIG. 24A

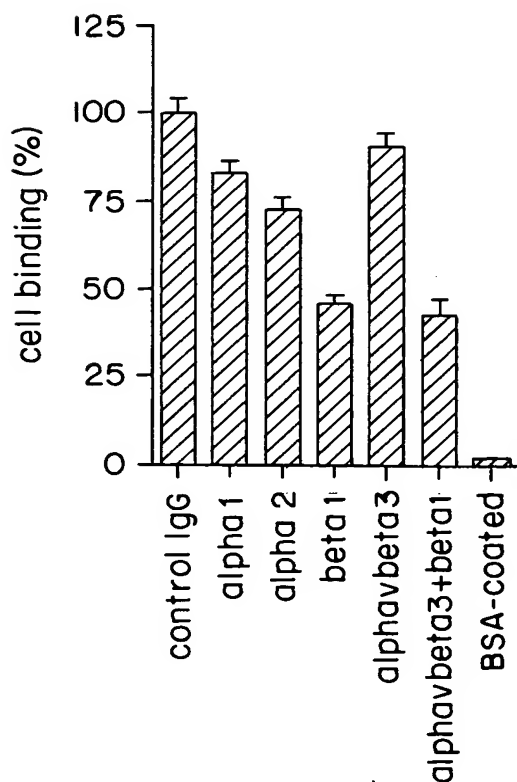


FIG. 24B

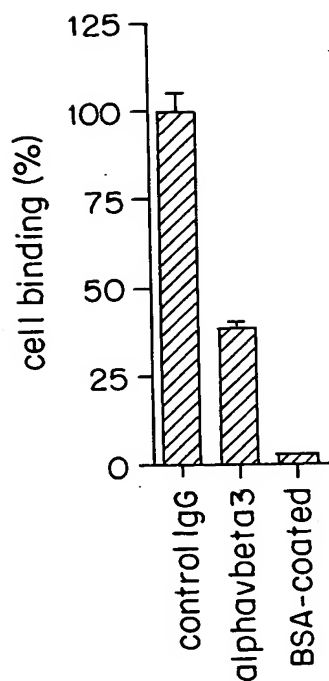


FIG. 24C

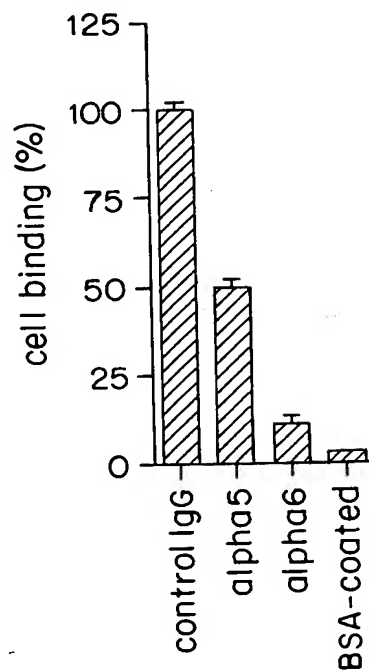


FIG. 24D

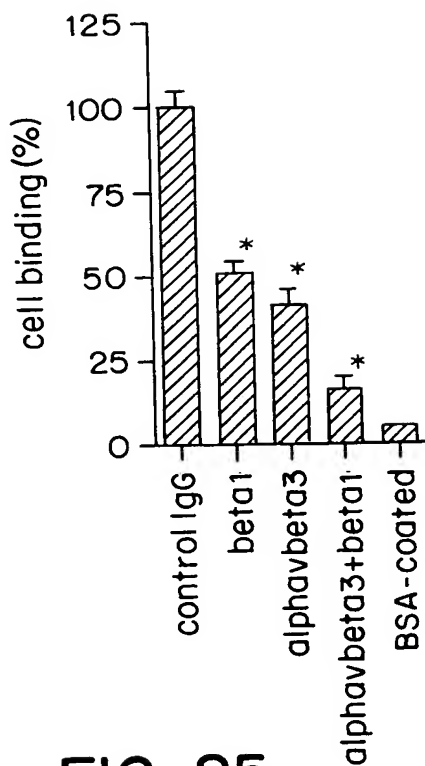


FIG. 25

FIG. 26

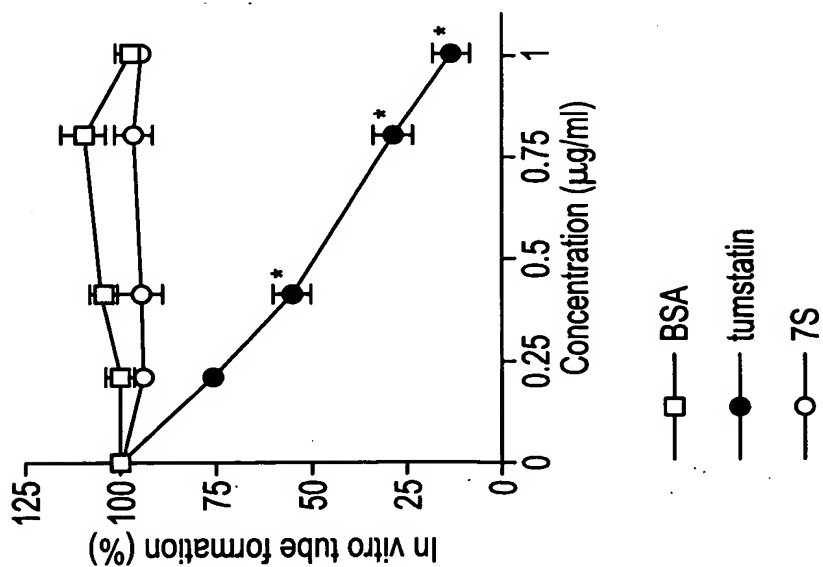


FIG. 27A

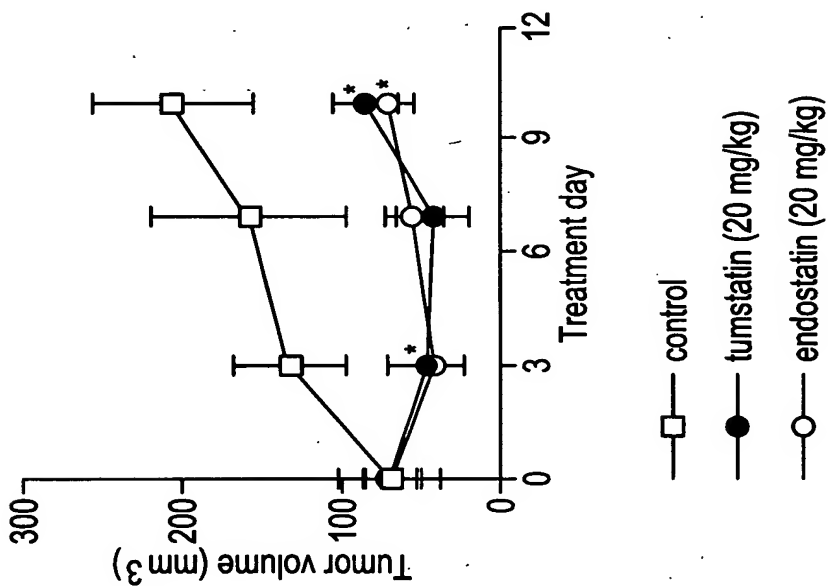


FIG. 27B

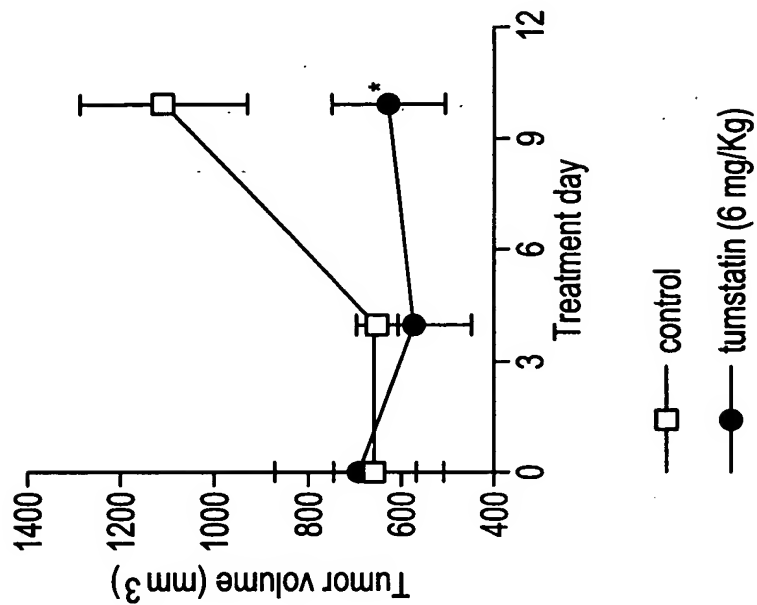
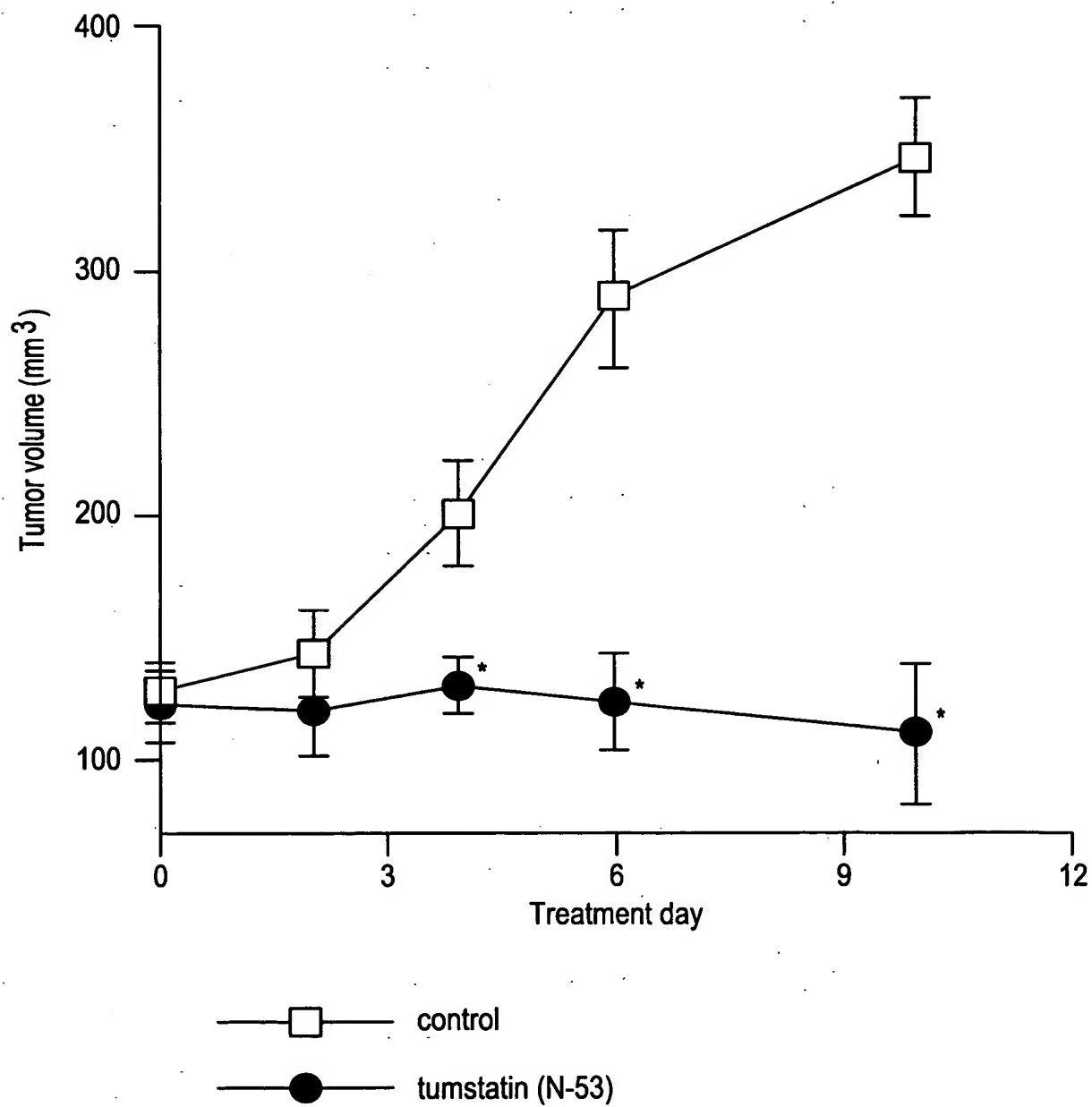


FIG. 28



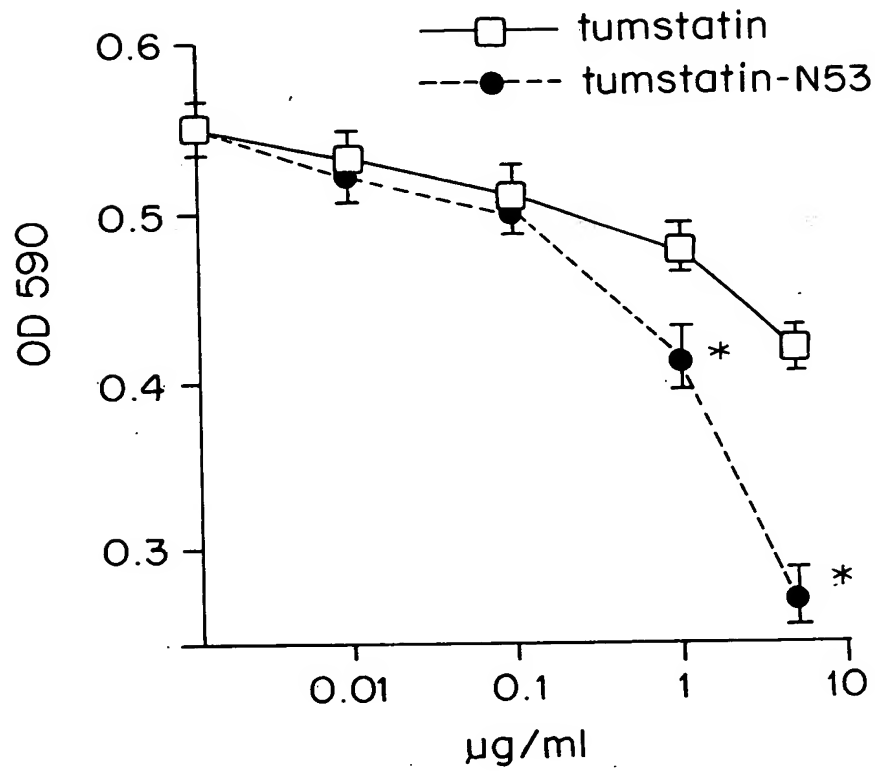
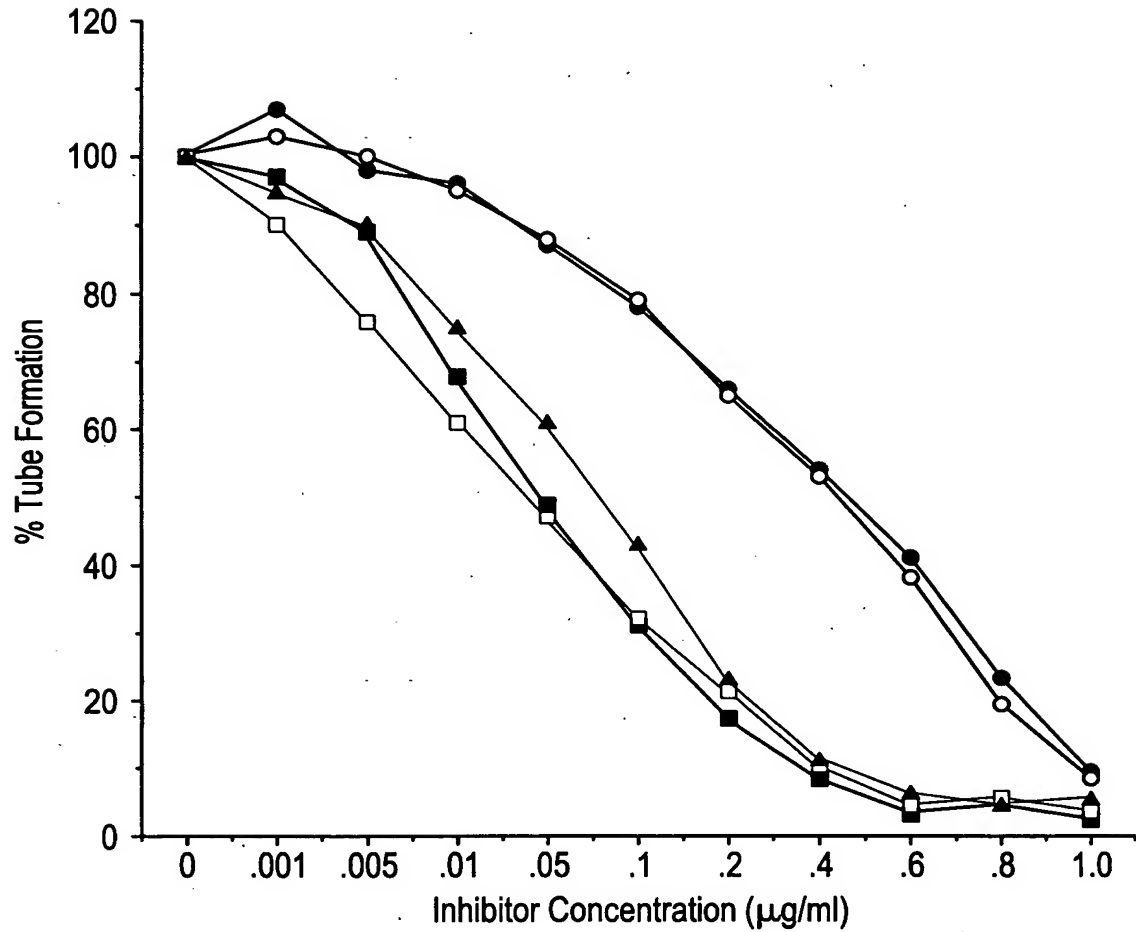


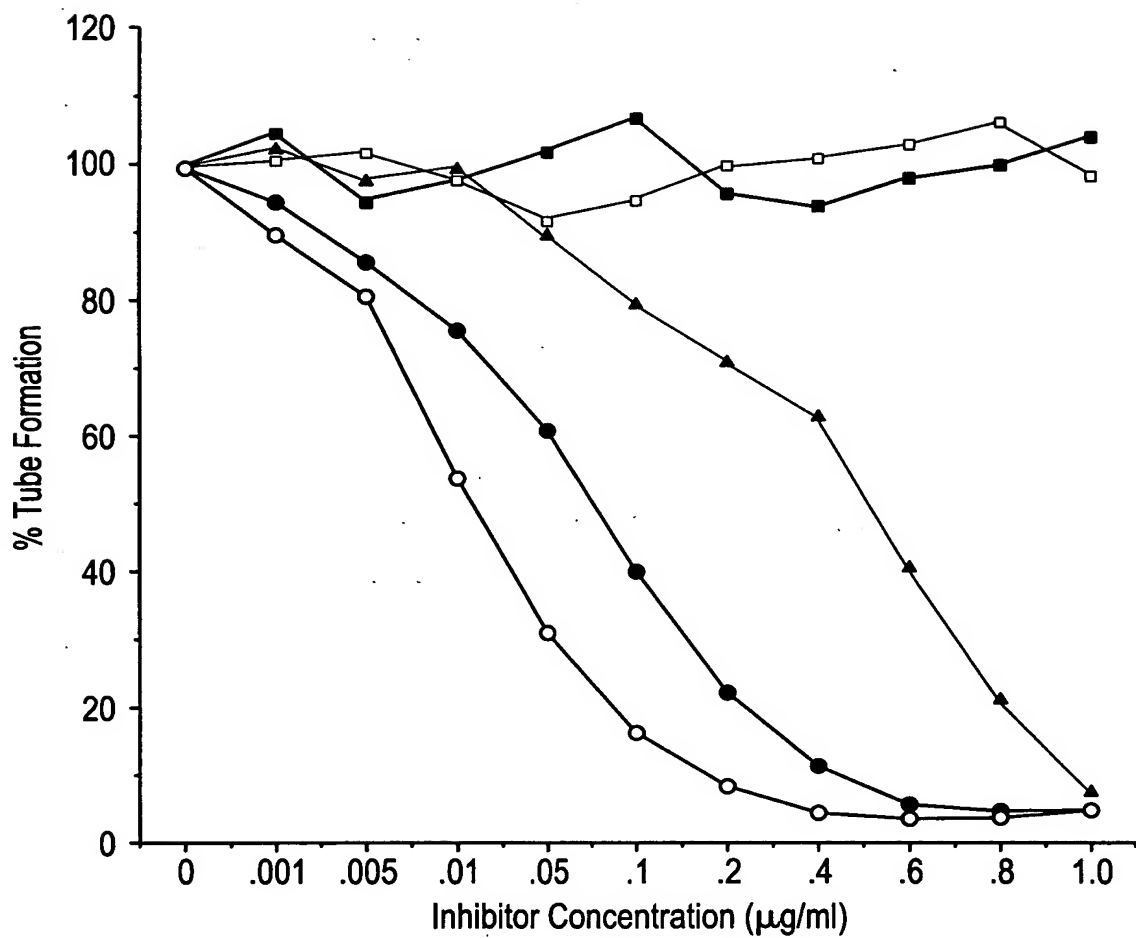
FIG. 29

FIG. 30

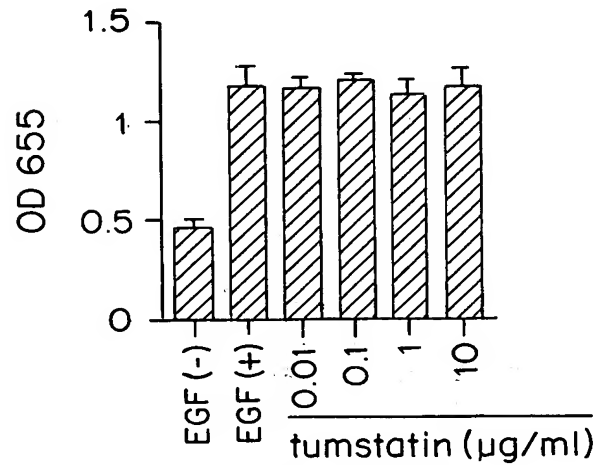
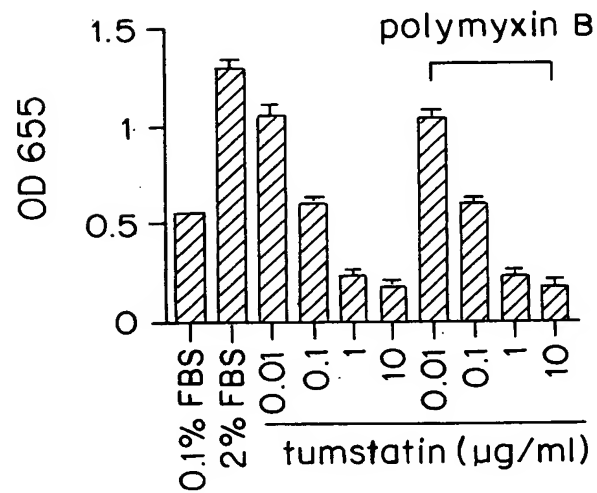
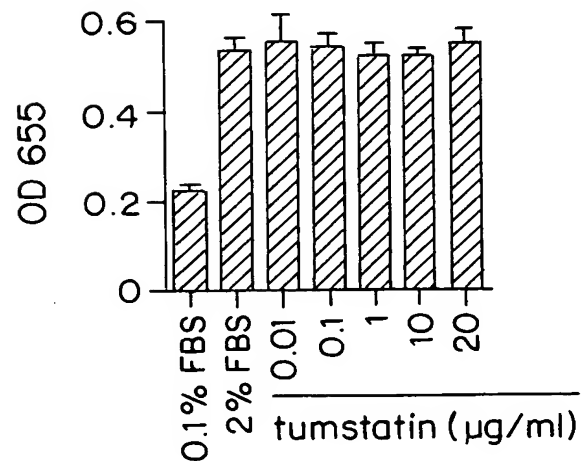


- Arresten
- Canstatin
- 12 kDa fragment of Arresten
- 8 kDa fragment of Arresten
- ▲— 10 kDa fragment of Canstatin

FIG. 31



- Tumstatin Fragment 333
- Tumstatin Fragment 334
- BSA
- α6
- ▲ Tumstatin

**FIG. 32A****FIG. 32B****FIG. 32C**

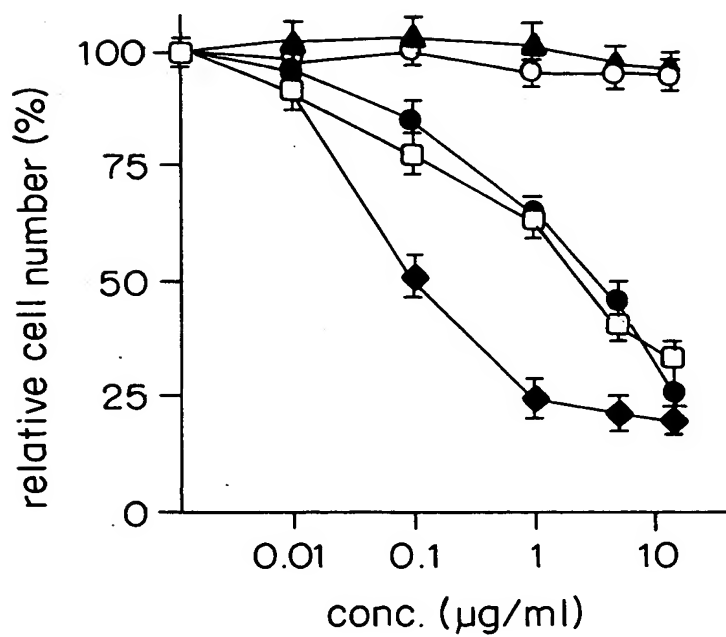


FIG. 33A

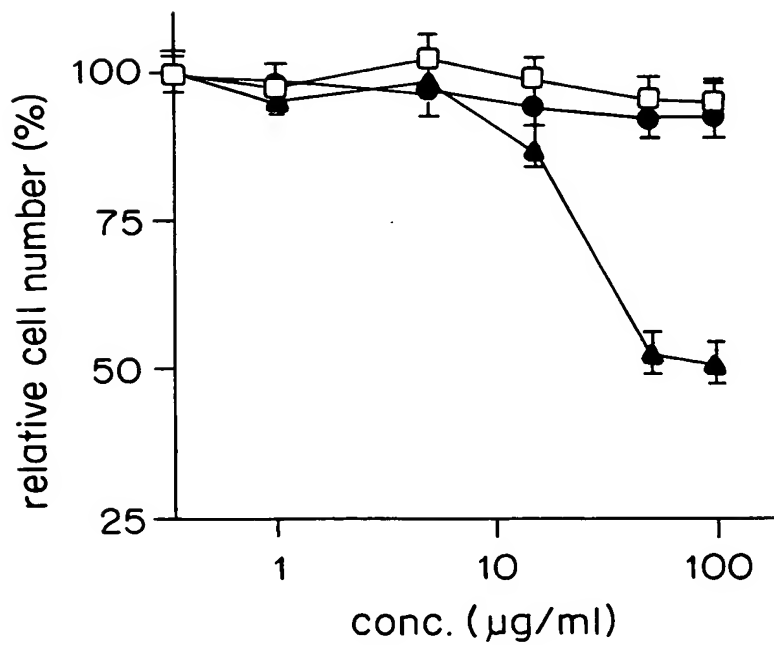


FIG. 33B

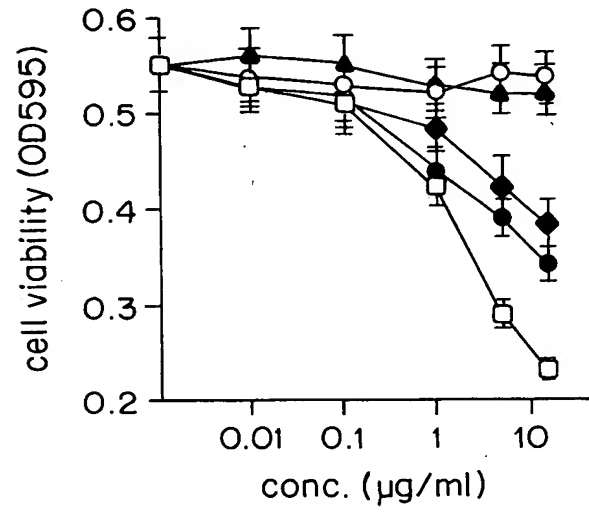


FIG. 34A

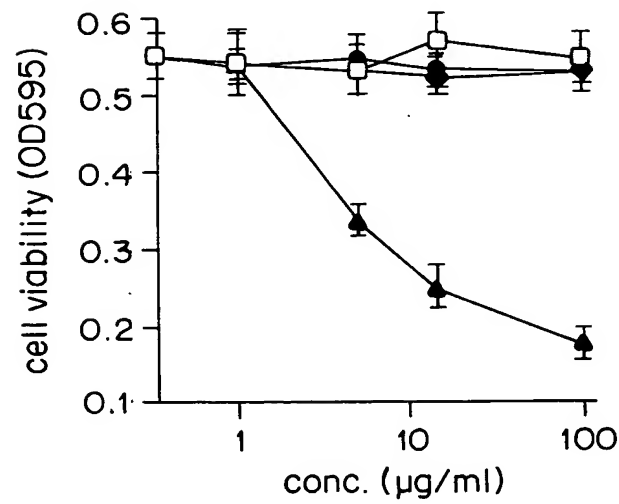


FIG. 34B

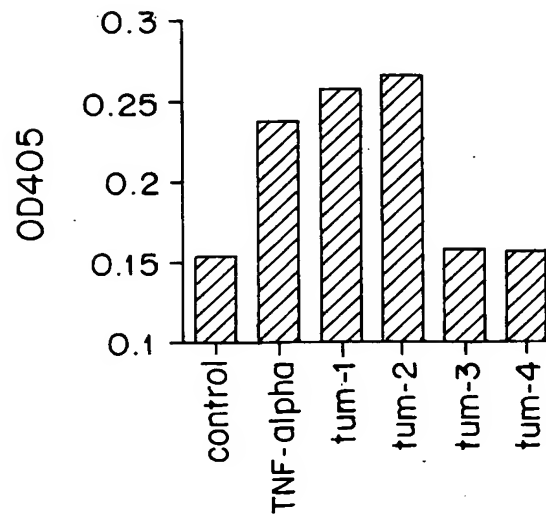


FIG. 35

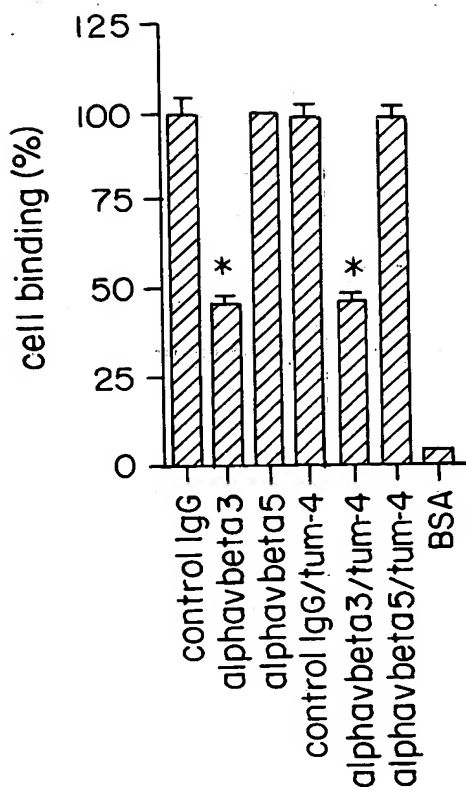


FIG. 36A

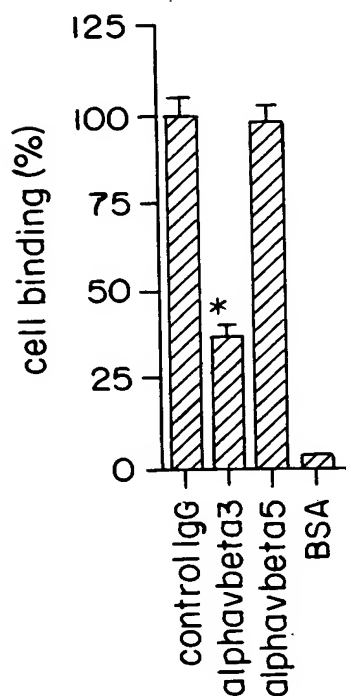


FIG. 36C

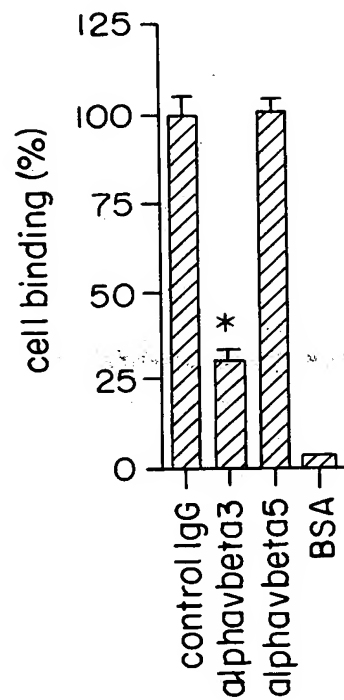


FIG. 36B

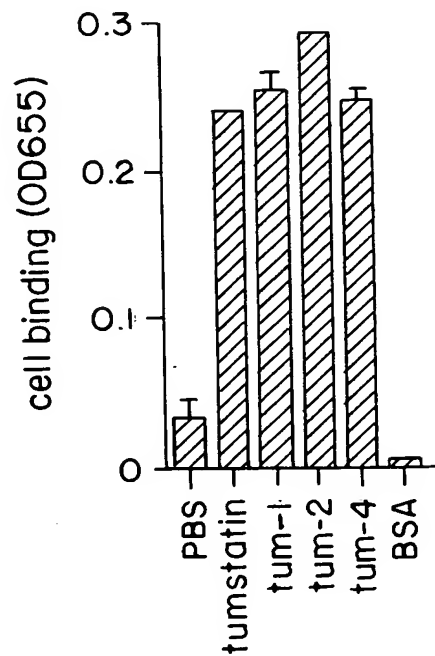


FIG. 37

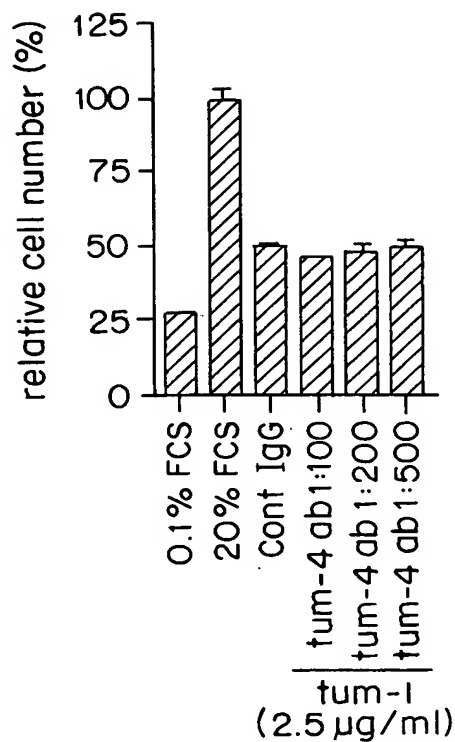


FIG. 38A

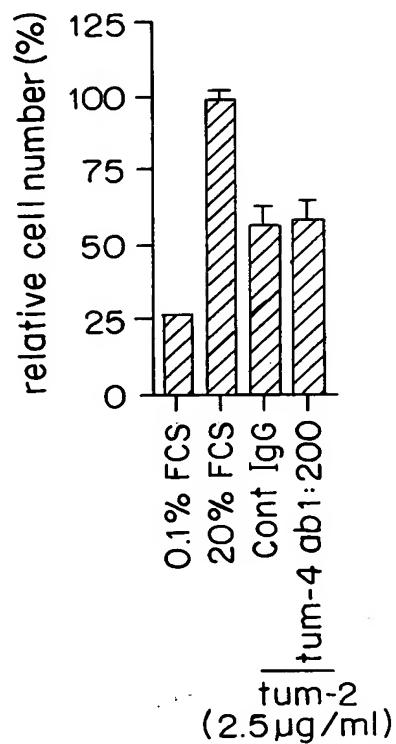


FIG. 38B

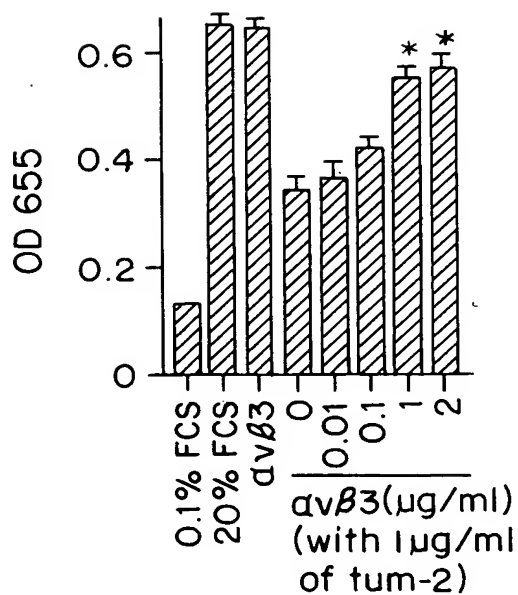


FIG. 38C

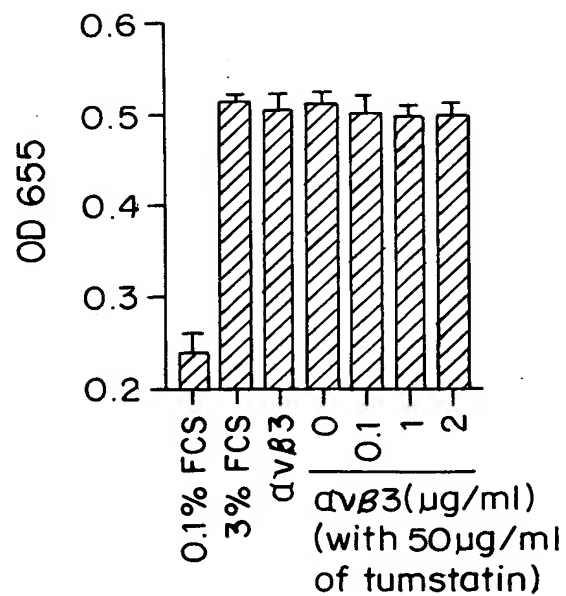


FIG. 38D

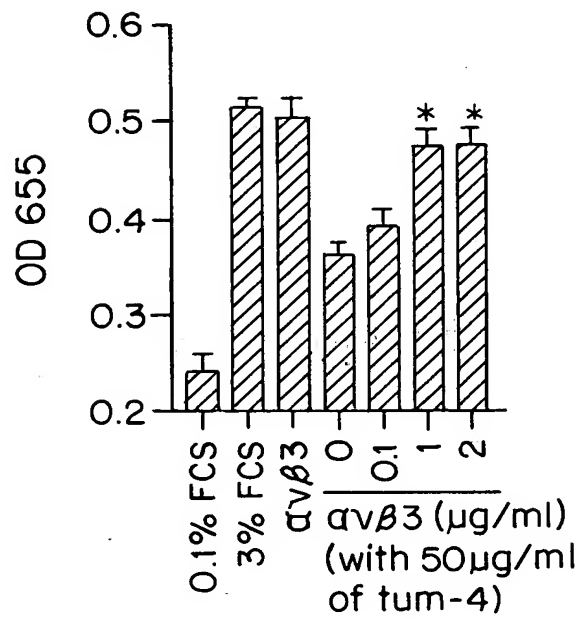


FIG. 38E

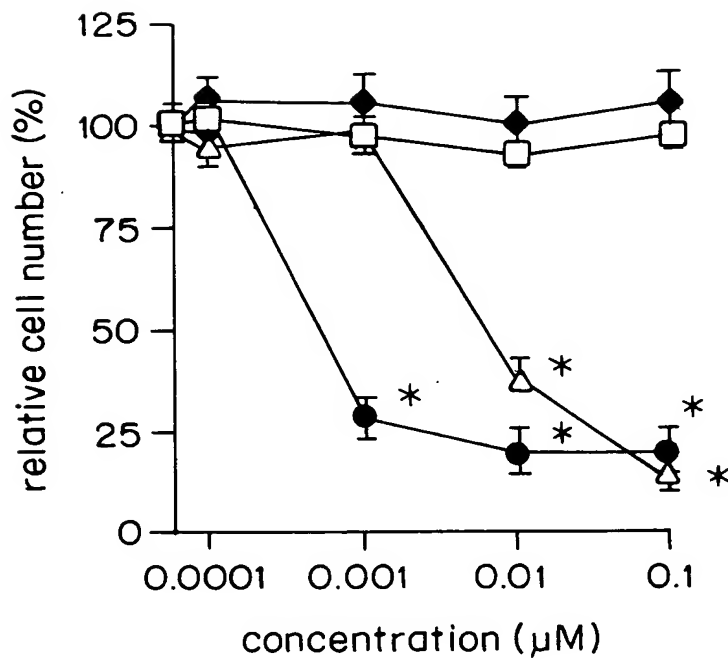


FIG. 39

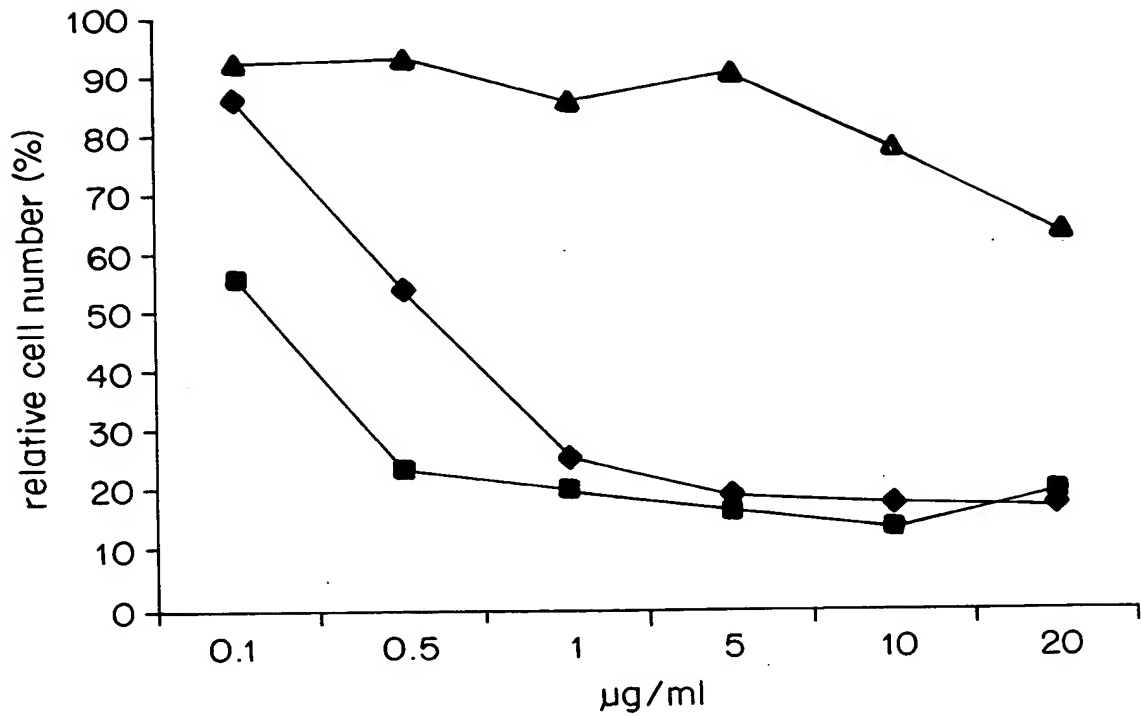


FIG. 40

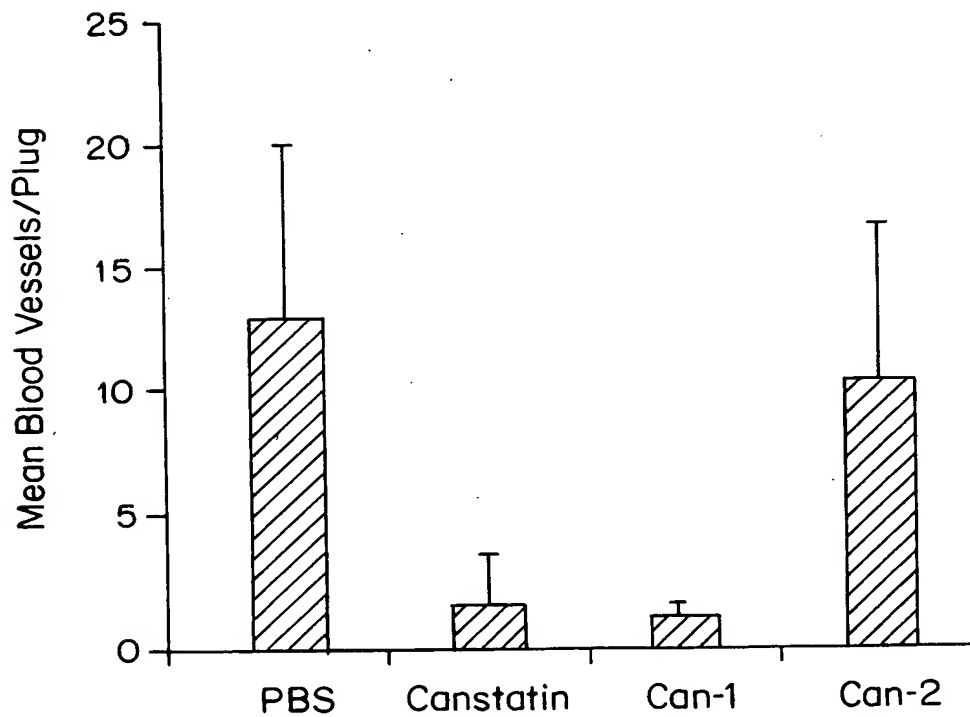


FIG. 41

FIG. 42

T1 GP-A
PGLKGK**RGD**SGSPATWTTTRGFVFTRHSQTT**TAIP**SCPEGTVPLY

T2 T3 T4
SGFSFLFVQGNQRAHGQDLGTLGSC LQRFTTMPFLFCNVNDVC

T3 T5 T6
T4 NFASRNDYSYWLSTPALMPMNMAPITGRALEPYISRCTVCEGP

T6 GP-B
AIAIAVHSQTT**DI****PPCP**HGWISLWKGFSFIMFTSAGSEGTGQA
LASPGSCLEEFRA SPFLECHGRGTCNYYSNSYSFWLASLNPER
MFRKPI PSTVKAGELEKII SRCQVCMKKRH

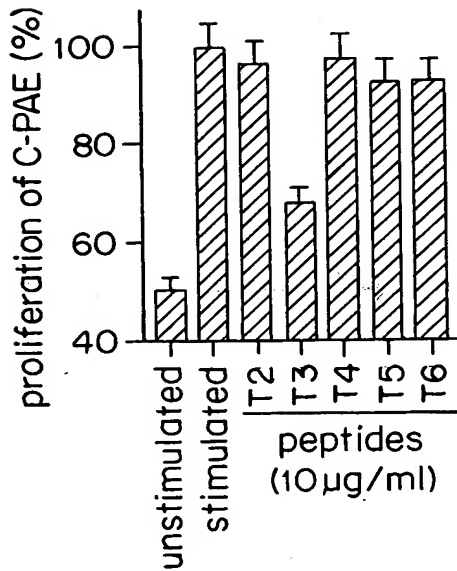


FIG. 43A

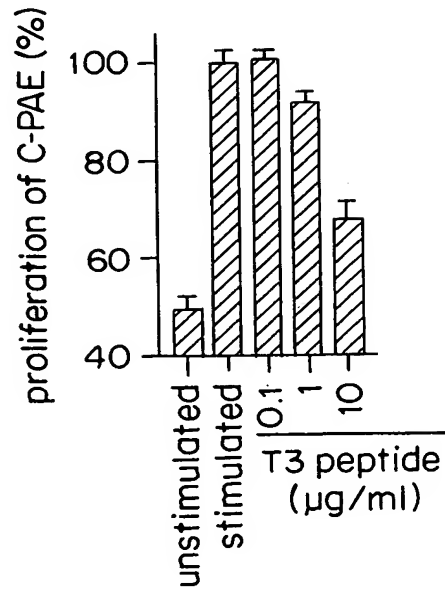


FIG. 43B

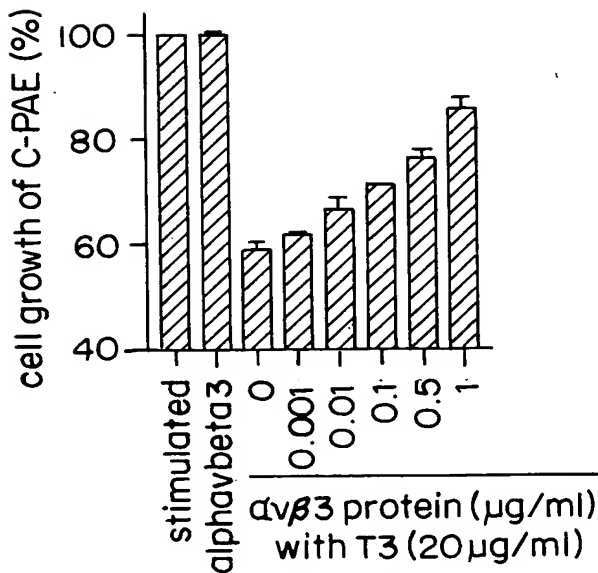


FIG. 43C

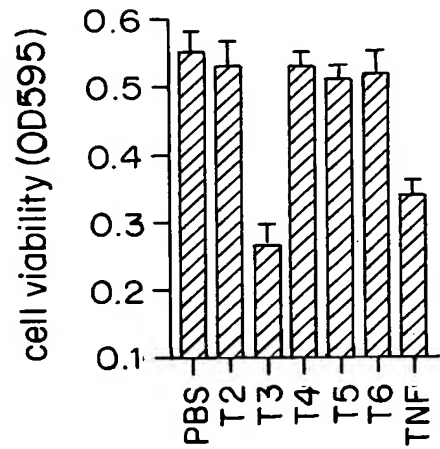


FIG. 43D

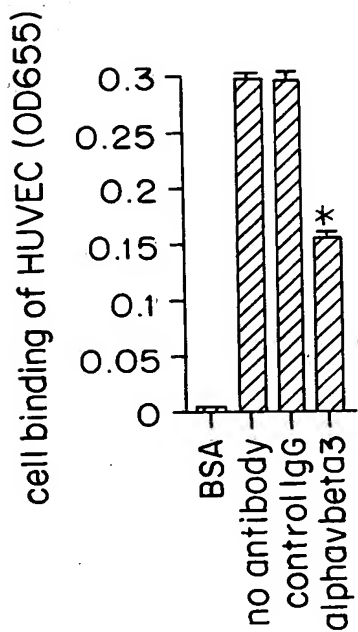


FIG. 44A

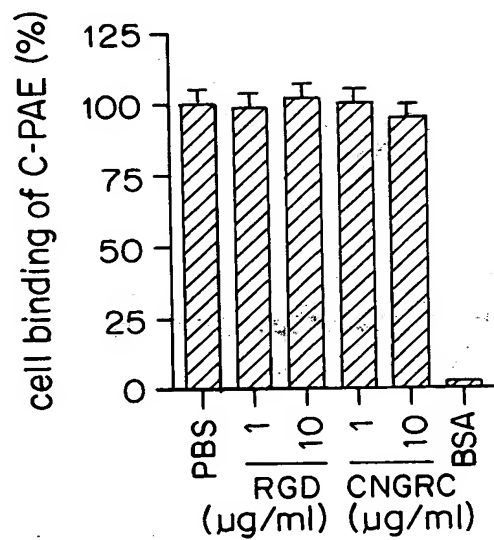


FIG. 44B

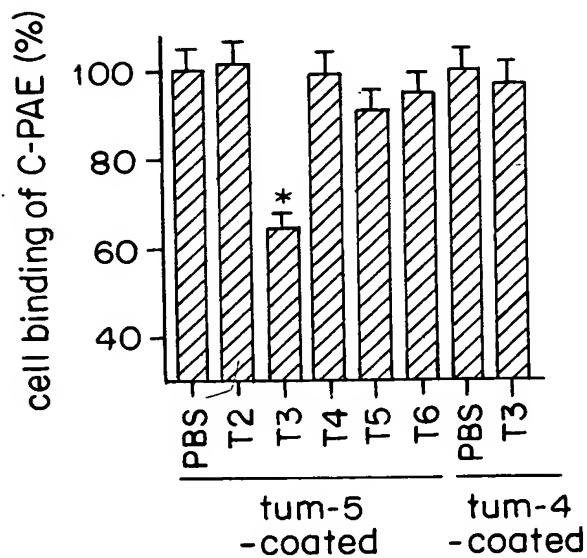


FIG. 44C

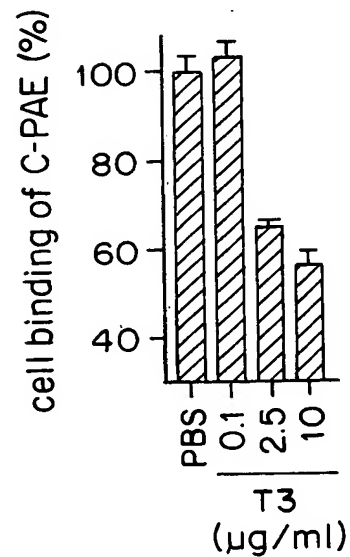


FIG. 44D

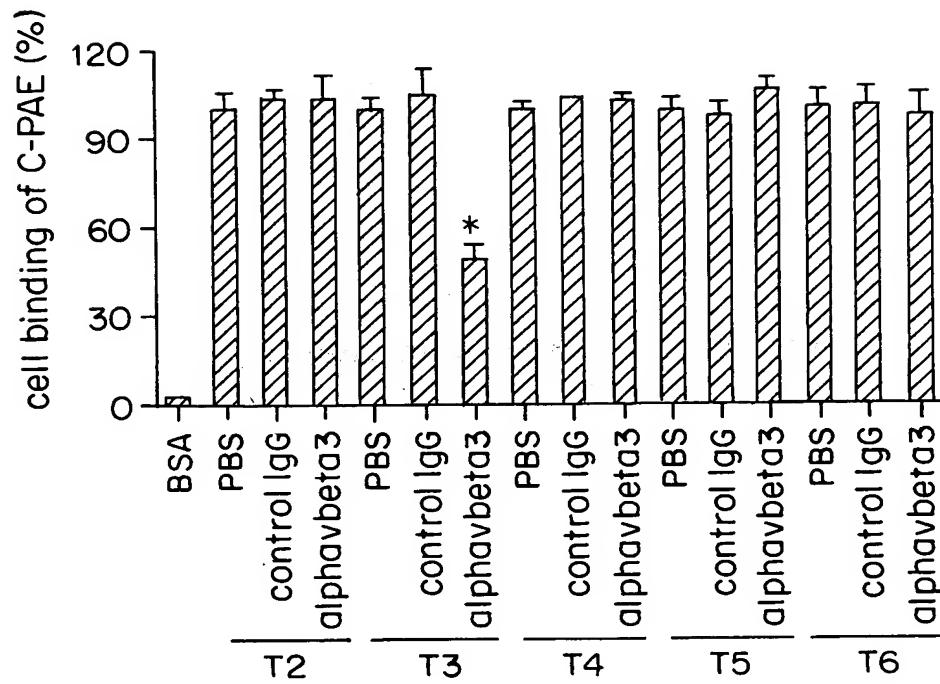


FIG. 44E

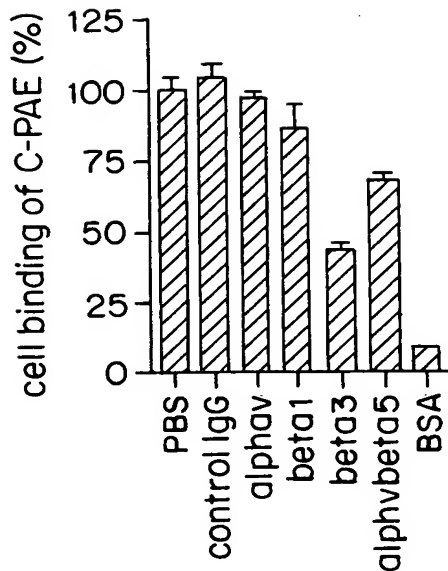


FIG. 44F

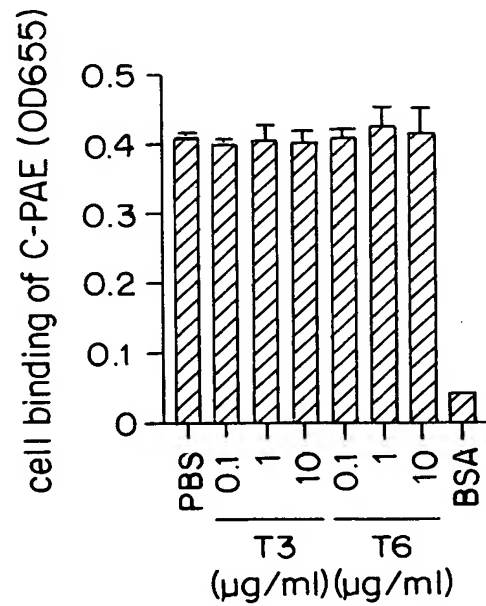


FIG. 44G

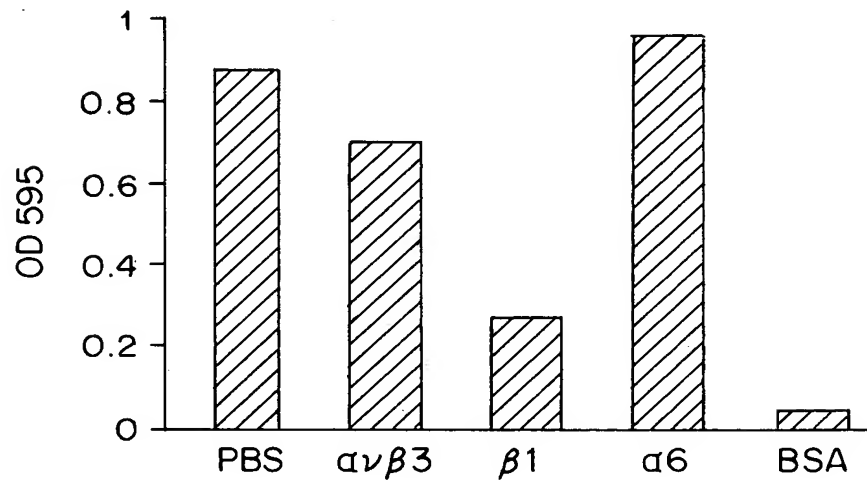


FIG. 45

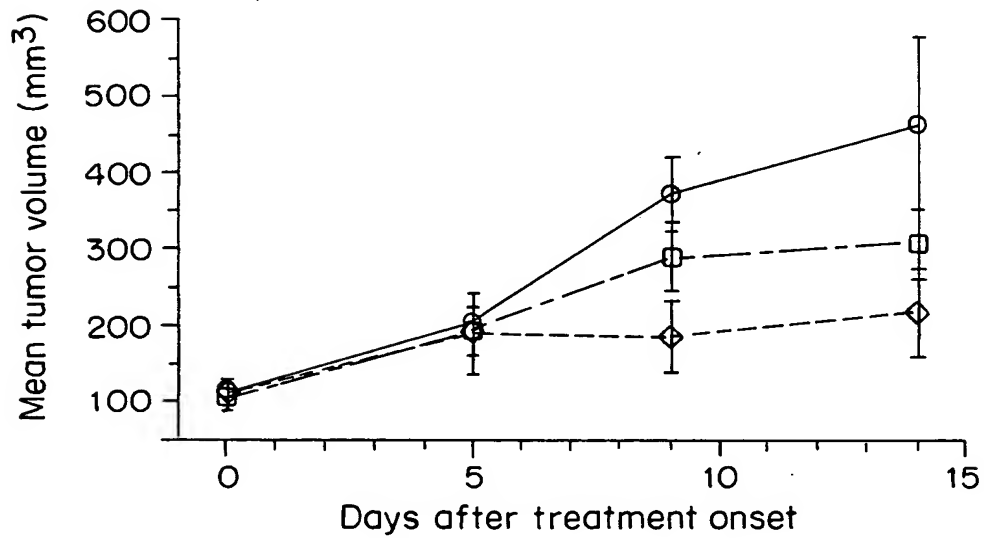


FIG. 46

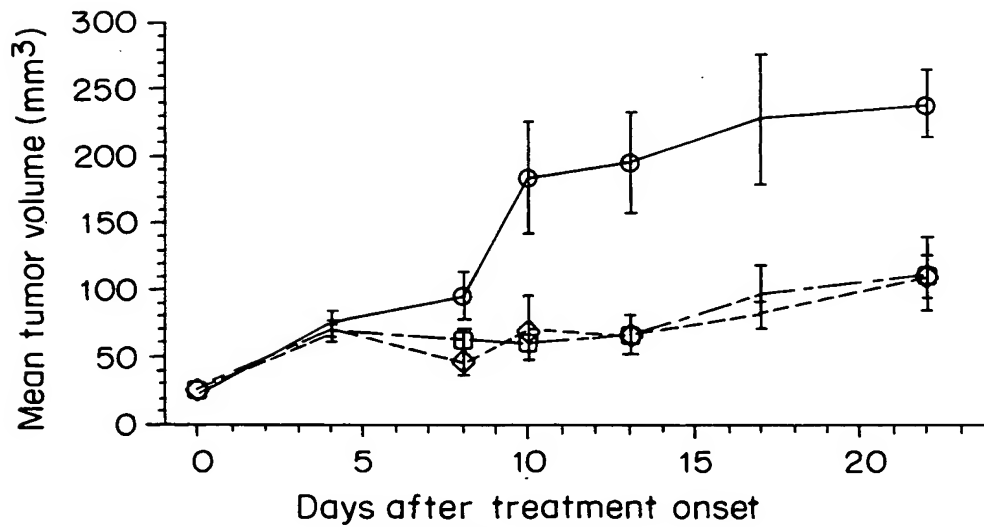


FIG. 47

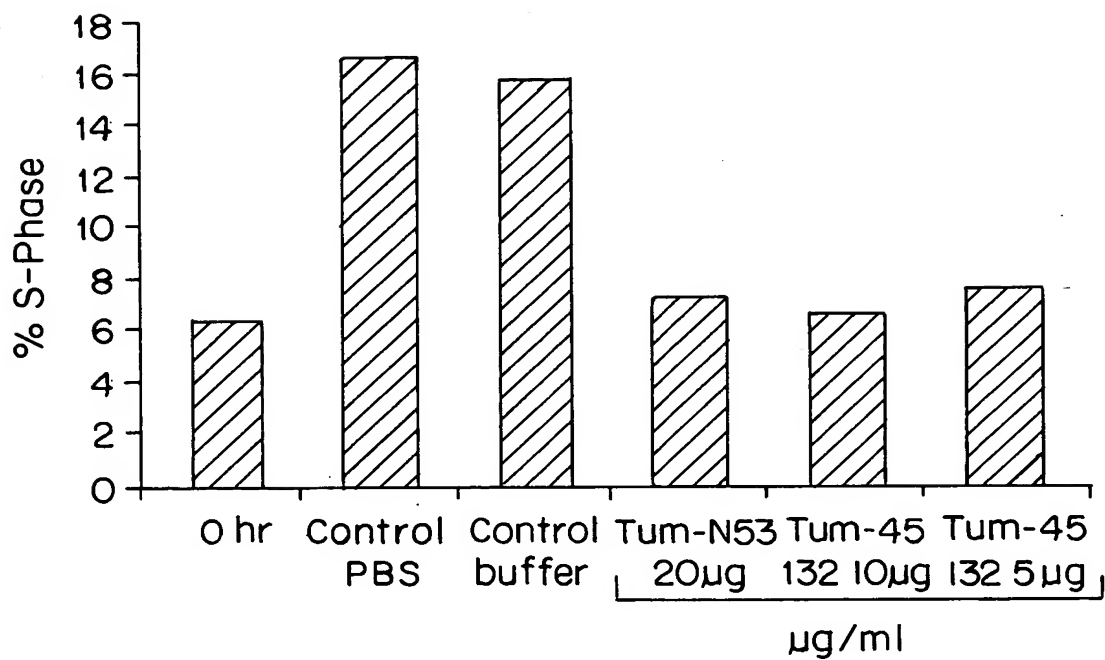


FIG. 48

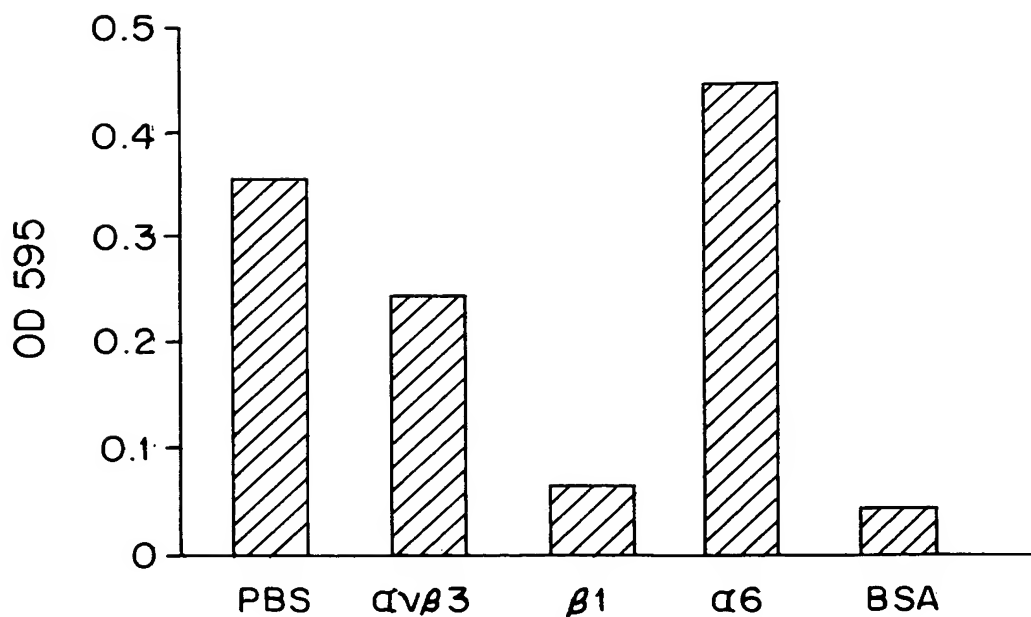


FIG. 49

Fig. 50

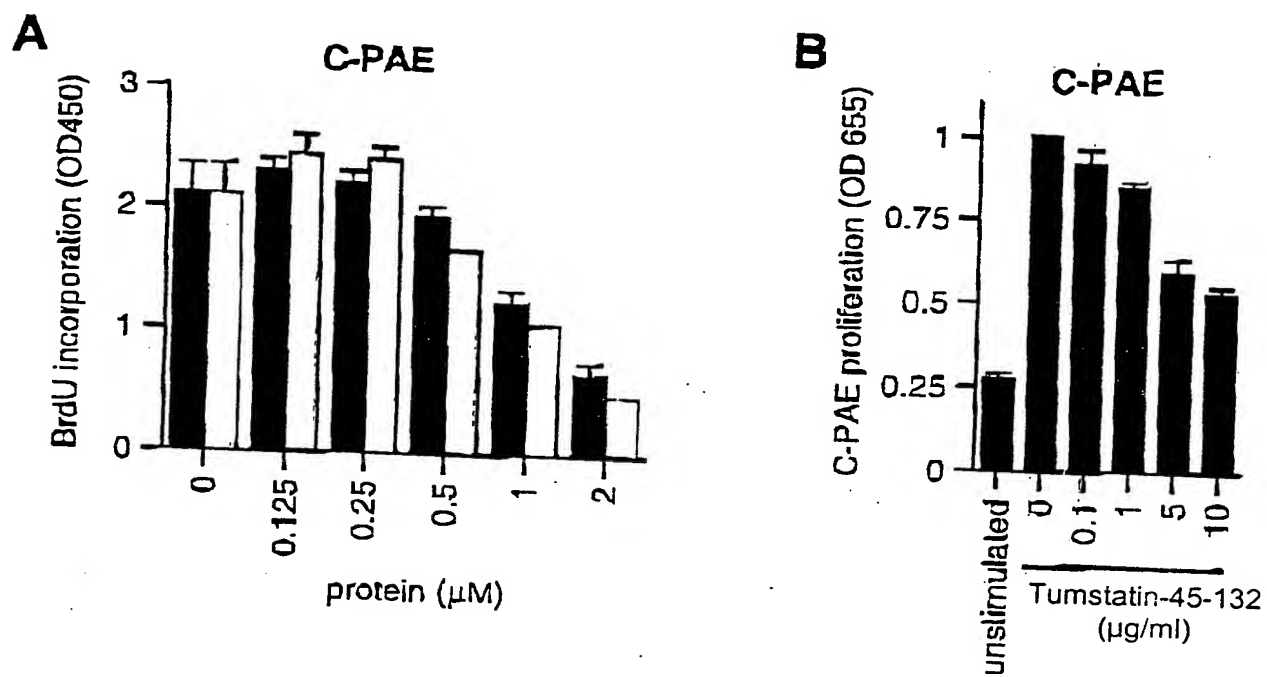


Fig. 51

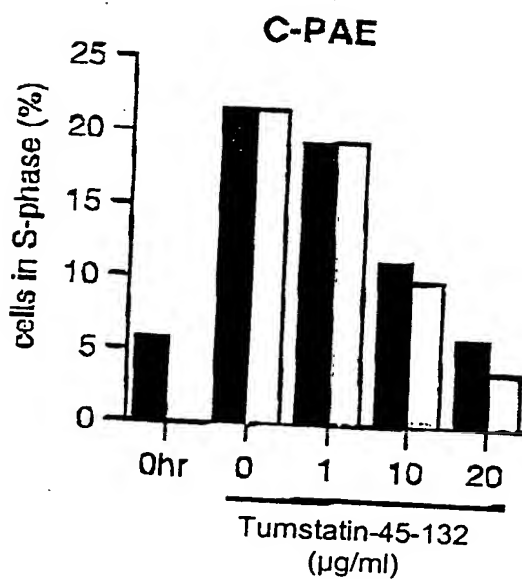


Fig. 52

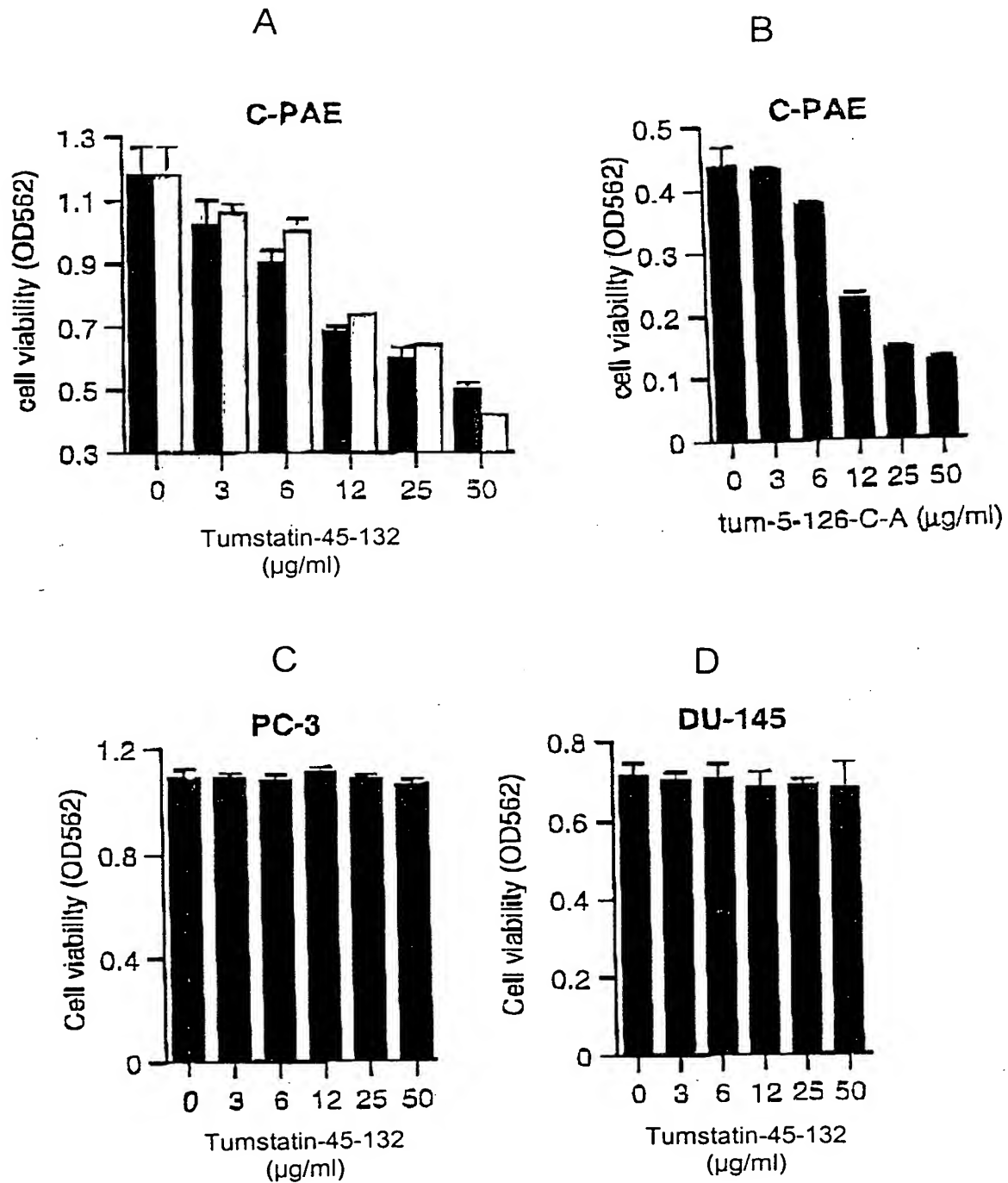


Fig. 54

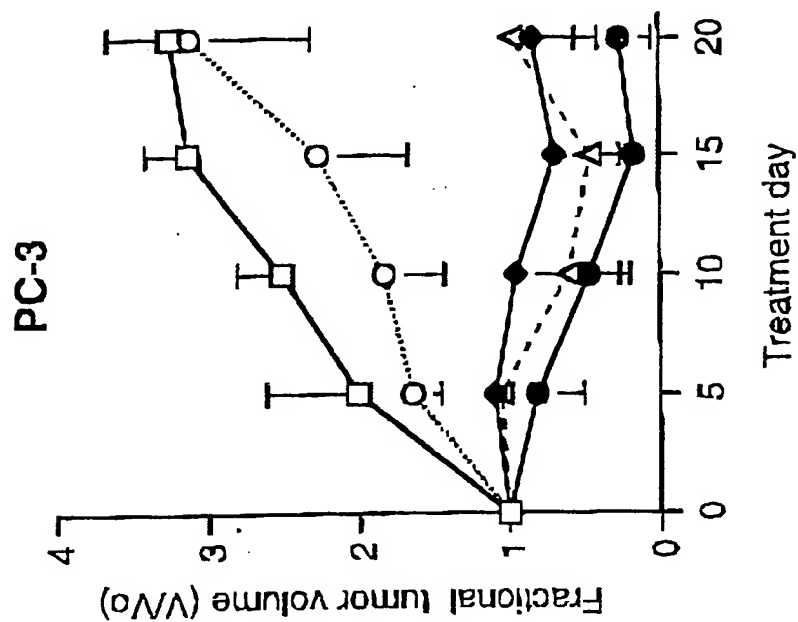
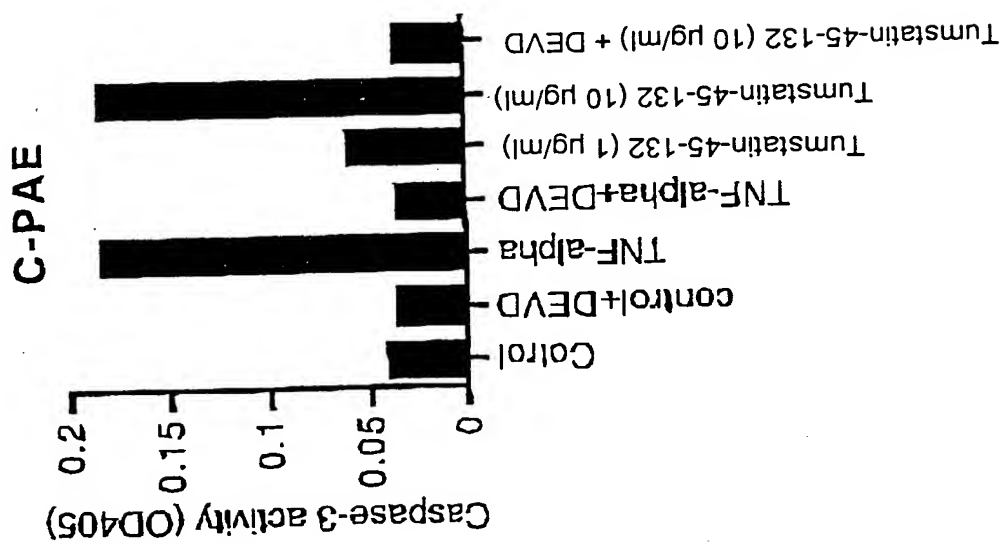


Fig. 53



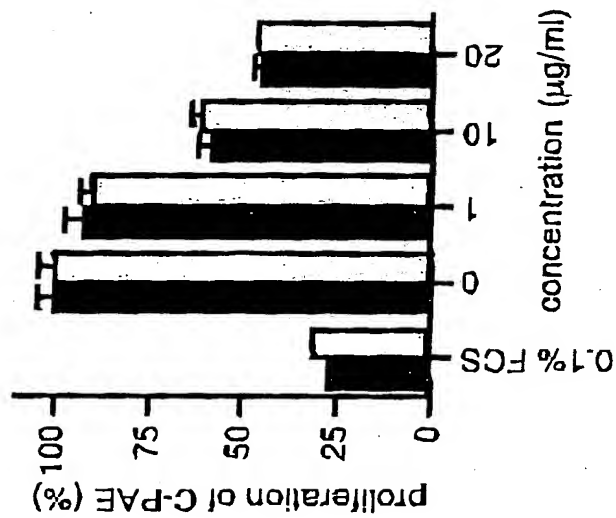


Fig. 56

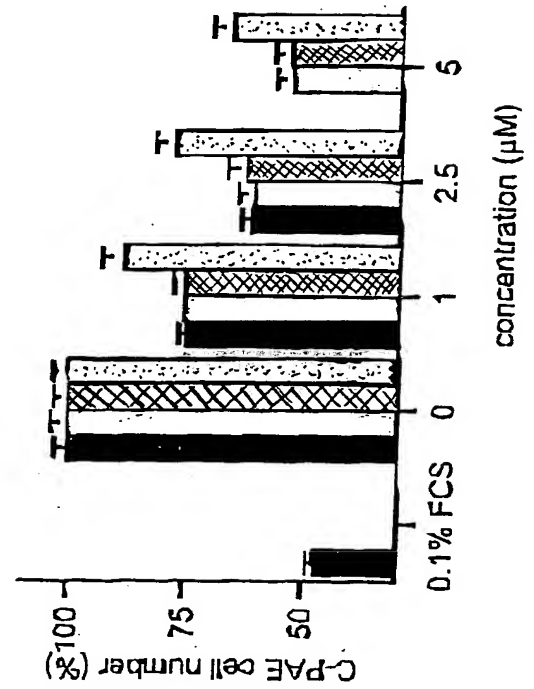


Fig. 57

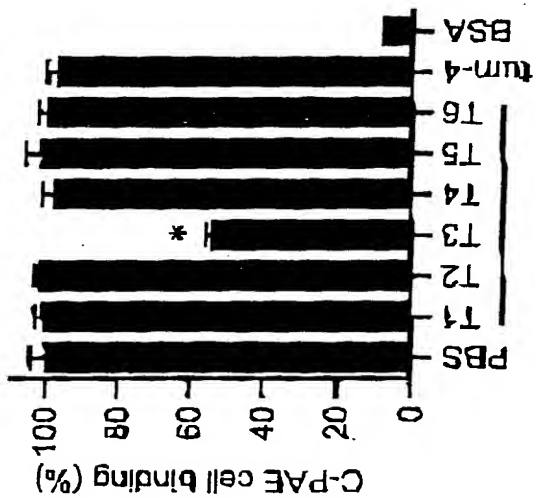


Fig. 55A



Fig. 55B

Fig. 58A

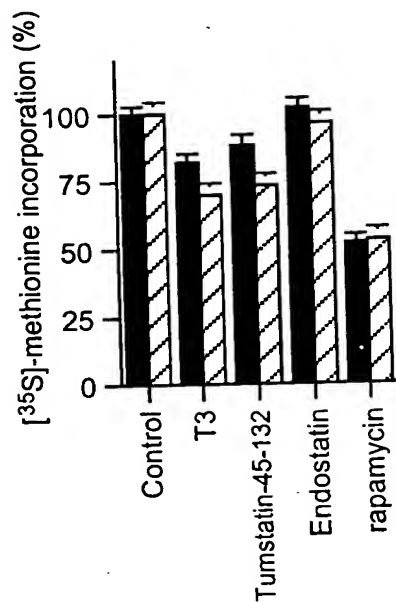


Fig. 58B

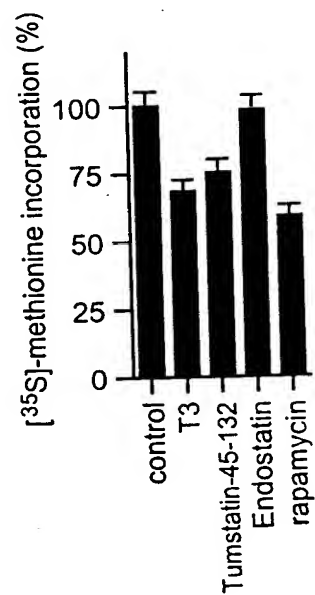


Fig. 58C

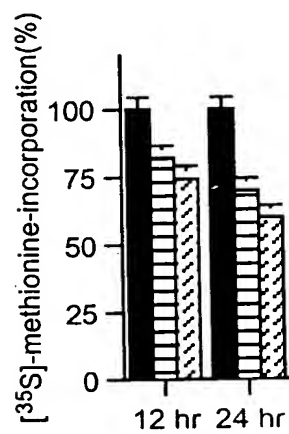


Fig. 58D

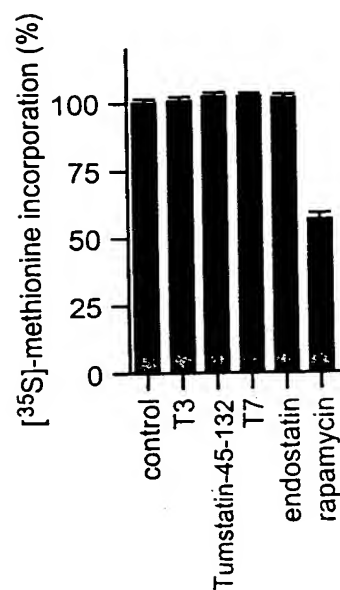


Fig. 58E

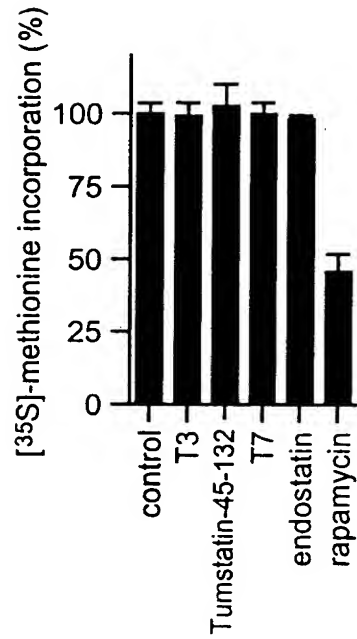


Fig. 58F

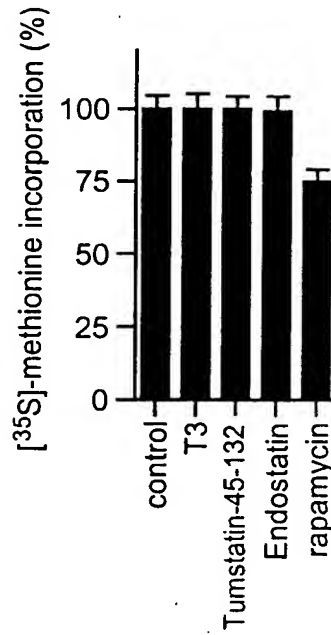


Fig. 58G

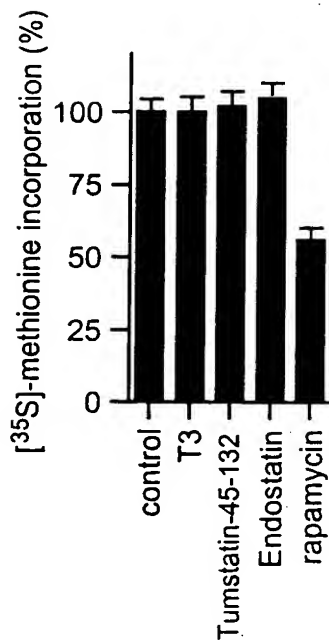


Fig. 58H

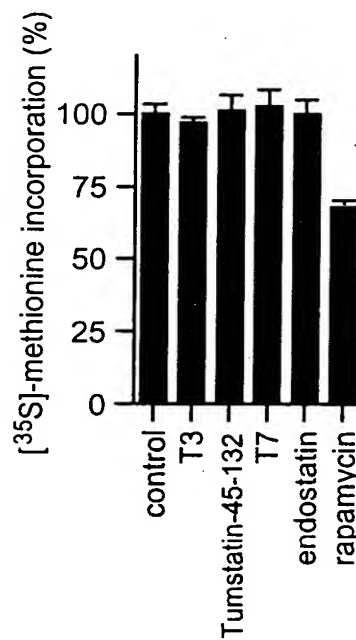


Fig. 59A

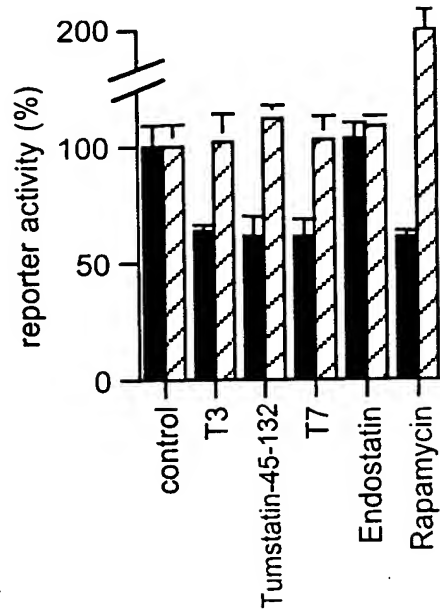


Fig. 59B

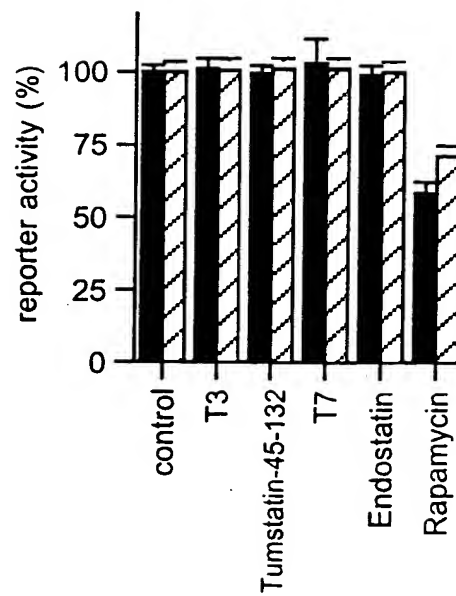


Fig. 60A

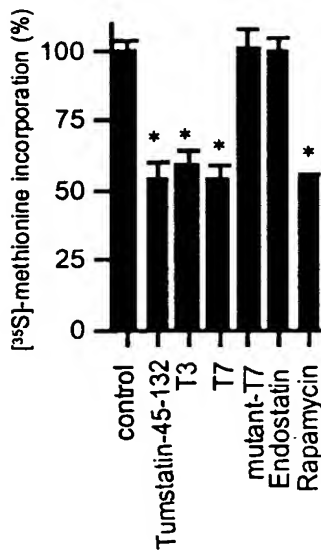


Fig. 60B

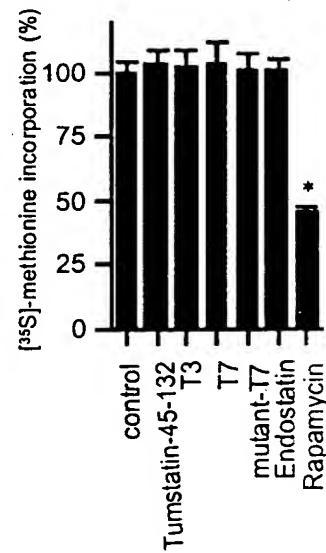


Fig. 60C

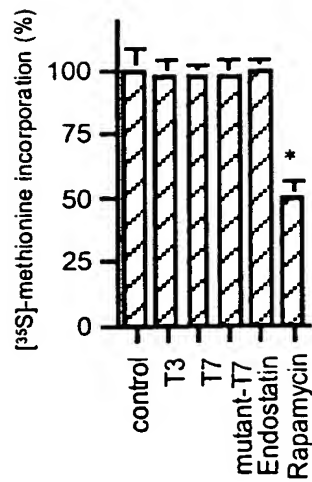


Fig. 60D

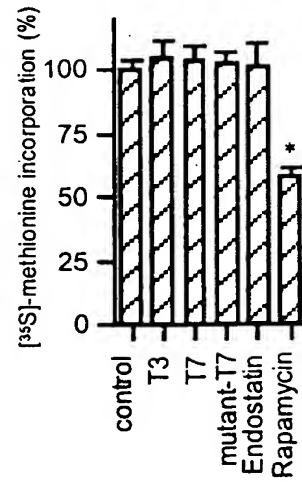


Fig. 60E

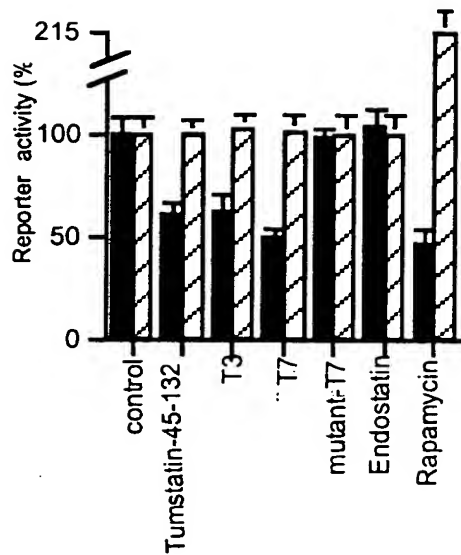


Fig. 60F

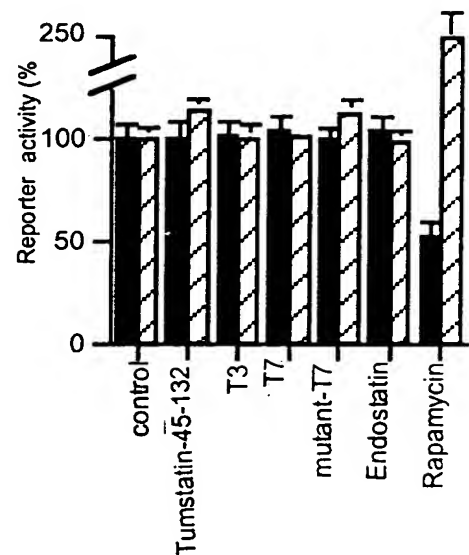


Fig. 60G

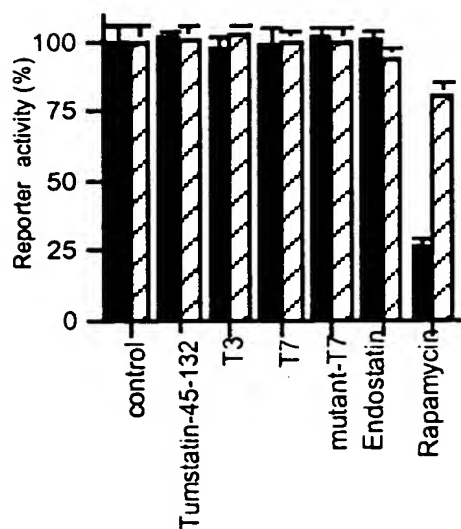


Fig. 60H

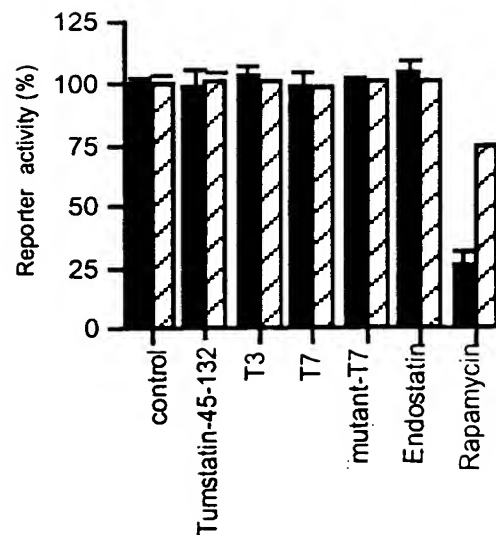


Fig. 61A

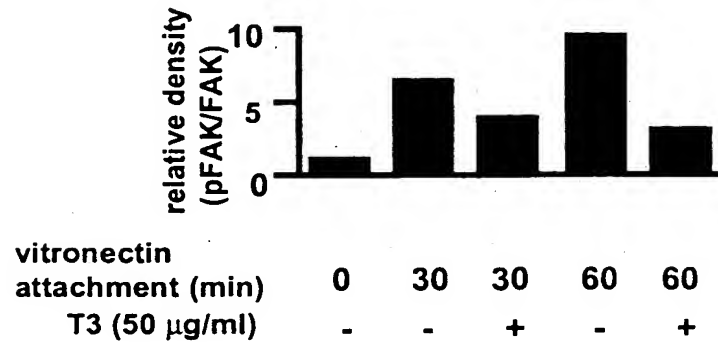


Fig. 61B

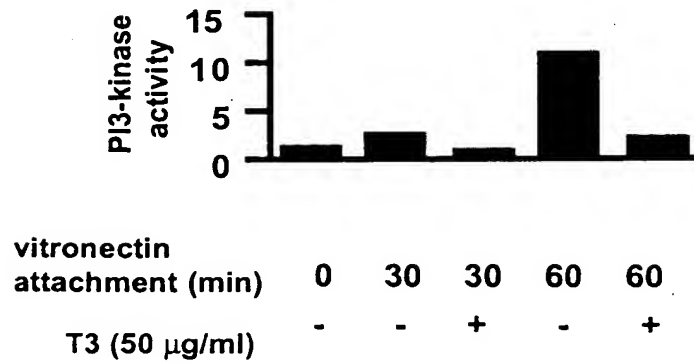


Fig. 61C

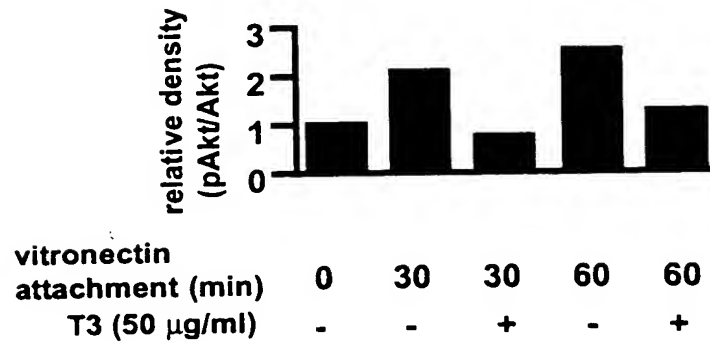


Fig. 61D

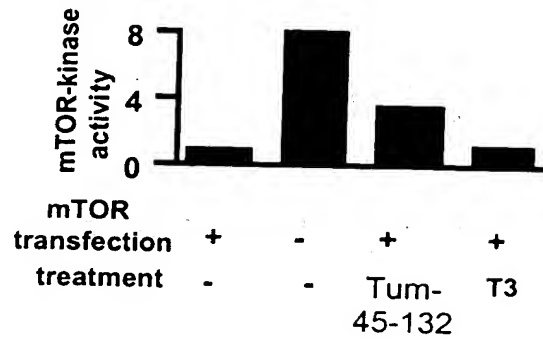


Fig. 61E

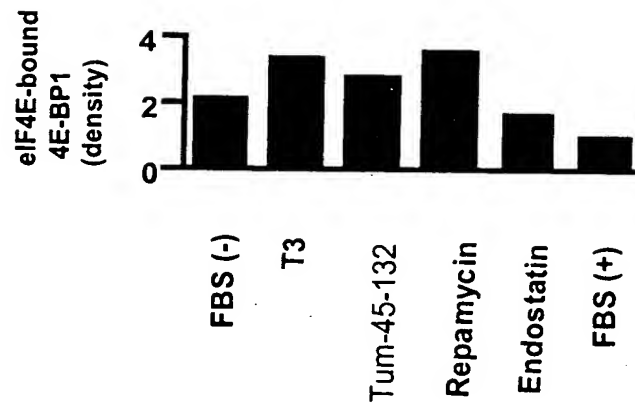
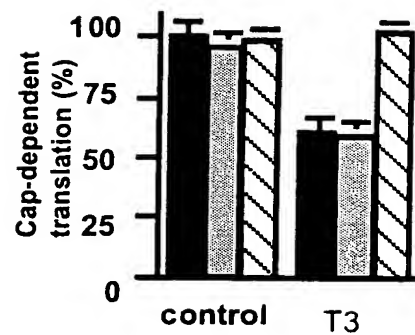


Fig. 61F



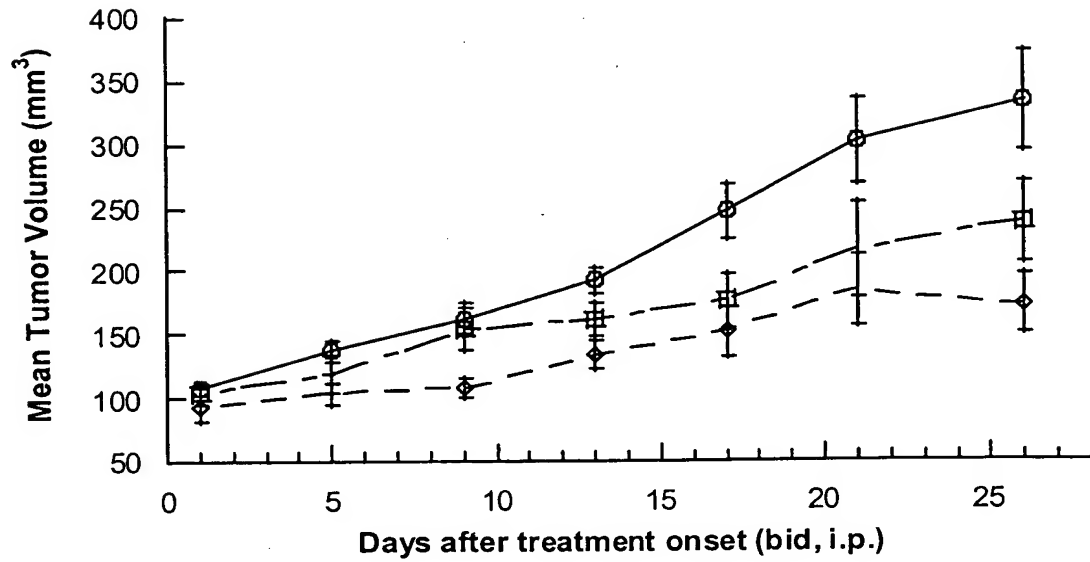


Fig. 62

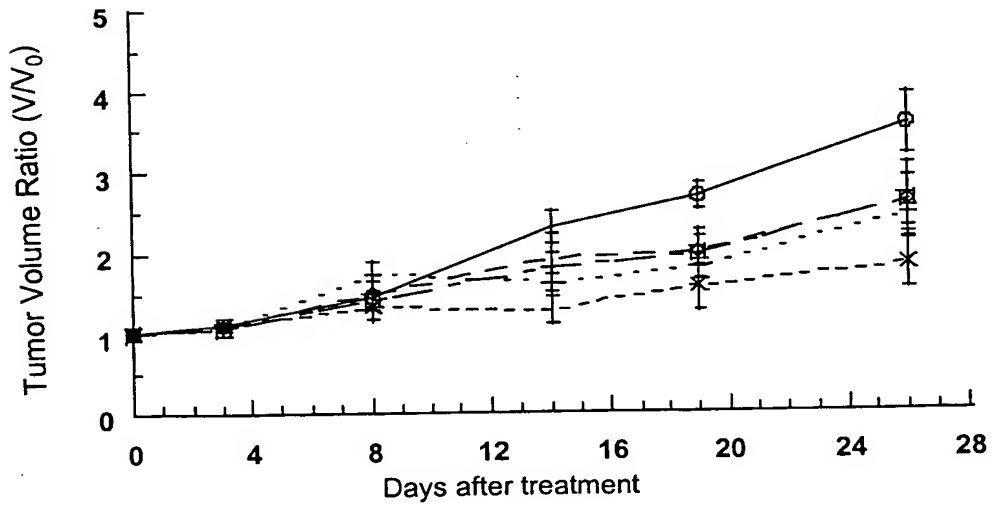


Fig. 63

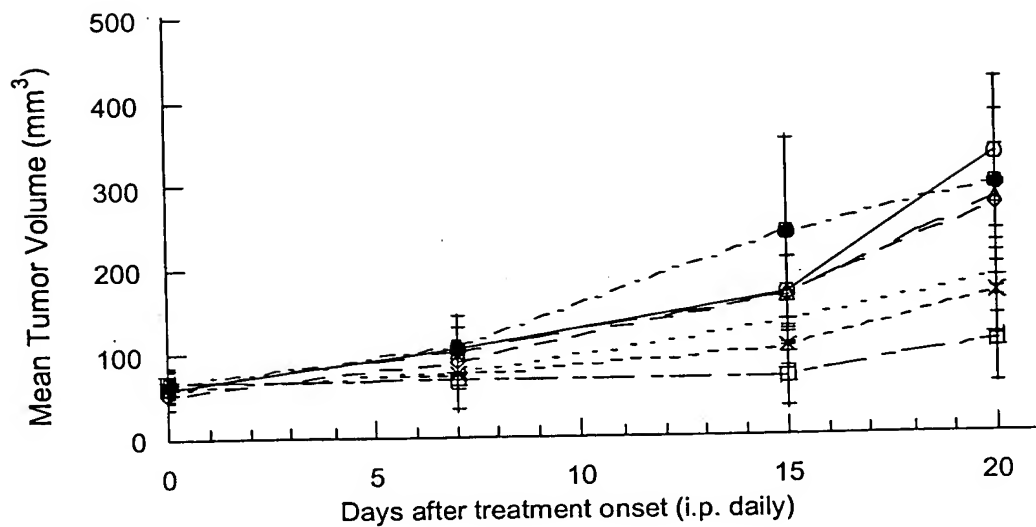


Fig. 64A

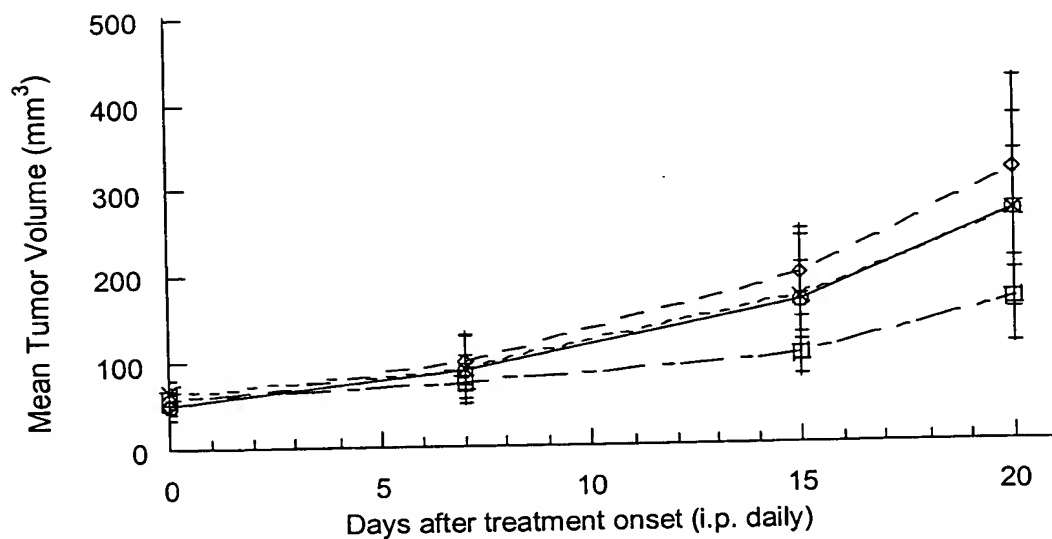


Fig. 64B

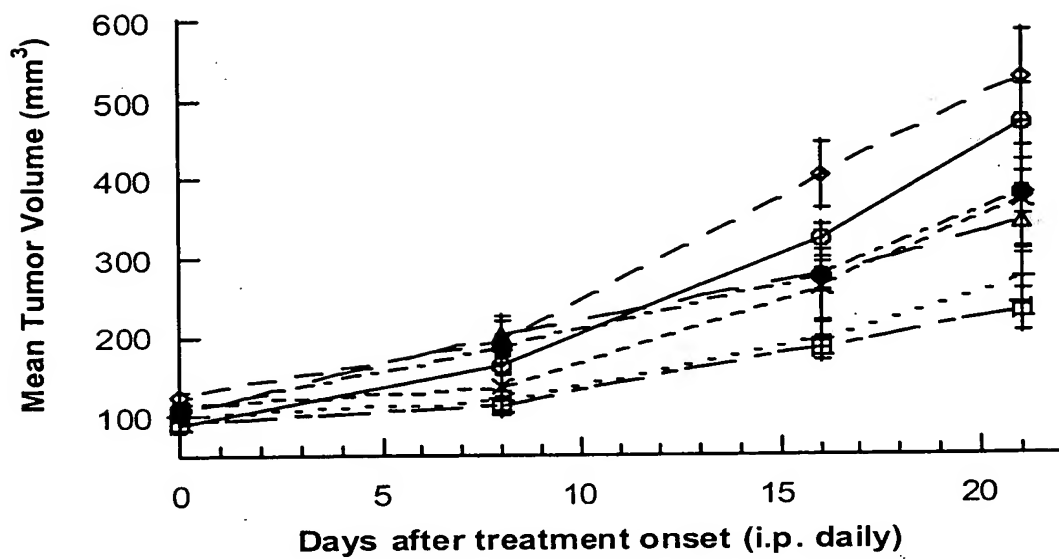


Fig. 65A

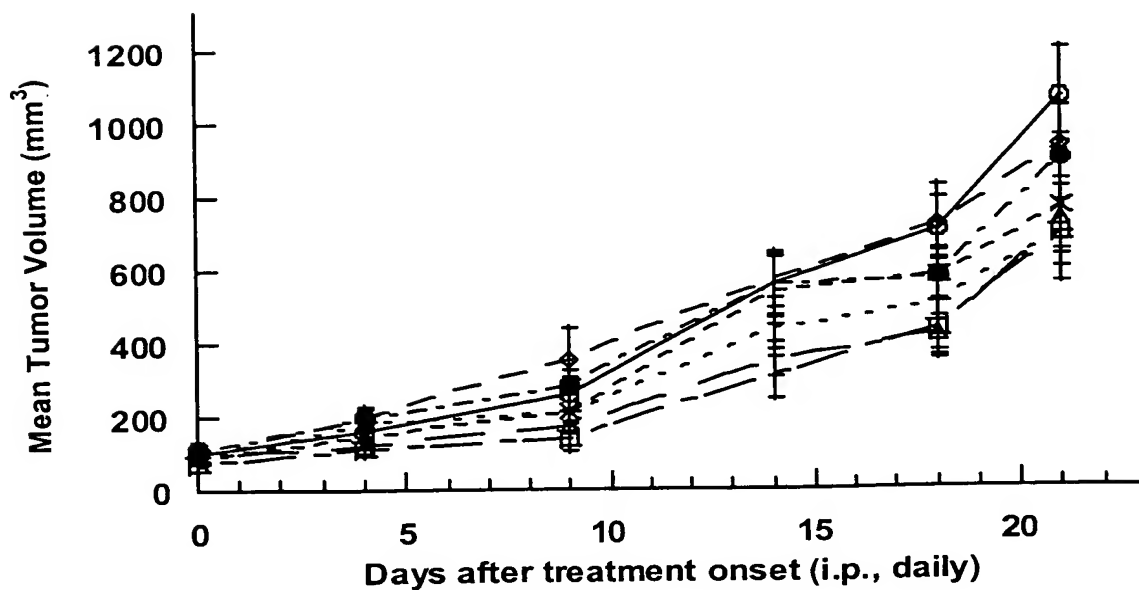


Fig. 65B